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Docket Number	10271-085-888	Type a plus sign (+) inside this box	+

INVENTOR(s) APPLICANT(s)

LAST NAME	FIRST NAME	MIDDLE INITIAL	RESIDENCE (CITY AND EITHER STATE OR FOREIGN COUNTRY)
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TITLE OF THE INVENTION (280 characters max)

EphA2 Vaccines

PENNIE & EDMONDS LLP
CORRESPONDENCE ADDRESS :20583

ENCLOSED APPLICATION PARTS (*check all that apply*)

☒ Specification	*Number of Pages*	107	☐ Applicant claims small entity status, see 37 CFR §1.27
☒ Drawing(s)	*Number of Sheets*	18	☒ Other (specify) Sequence Listing (67 Pages)

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Respectfully submitted,

Signature Anthony M. Insogna REGISTRATION NO. (*if appropriate*) 35,203 Date 12/24/03

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EphA2 VACCINES

1. FIELD OF THE INVENTION

[0001] The present invention relates to methods and compositions designed for the treatment, management, or prevention of proliferative cell disease. The present invention further relates to methods and compsitions for eliciting an immune response against hyperproliferative cells. The methods of the invention comprise the administration of an effective amount of an EphA2 vaccine, comprising, for example, EphA2 antigenic peptides or an EphA2 antigenic peptide expression vehicle. The invention also provides pharmaceutical compositions comprising one or more EphA2 antigenic peptides or peptide expression vehicles of the invention either alone or in combination with one or more other agents useful for therapy of proliferative disorders.

2. BACKGROUND OF THE INVENTION

2.1 HYPERPROLIFERATIVE DISEASES

2.1.1 Cancer

[0002] A neoplasm, or tumor, is a neoplastic mass resulting from abnormal uncontrolled cell growth which can be benign or malignant. Benign tumors generally remain localized. Malignant tumors are collectively termed cancers. The term "malignant" generally means that the tumor can invade and destroy neighboring body structures and spread to distant sites to cause death (for review, *see* Robbins and Angell, 1976, Basic Pathology, 2d Ed., W.B. Saunders Co., Philadelphia, pp. 68-122). Cancer can arise in many sites of the body and behaves differently depending upon its origin. Cancerous cells destroy the part of the body in which they originate and then spread to other part(s) of the body where they start new growth and cause more destruction.

[0003] More than 1.2 million Americans develop cancer each year. Cancer is the second leading cause of death in the United States and, if current trends continue, cancer is expected to be the leading cause of death by the year 2010. Lung and prostate cancer are the top cancer killers for men in the United States. Lung and breast cancer are the top cancer killers for women in the United States. One in two men in the United States will be diagnosed with cancer at some time during his lifetime. One in three women in the United States will be diagnosed with cancer at some time during her lifetime.

[0004] A cure for cancer has yet to be found. Current treatment options, such as surgery, chemotherapy and radiation treatment, are often either ineffective or present serious side effects.

2.1.2 Metastasis

[0005] The most life-threatening forms of cancer often arise when a population of tumor cells gains the ability to colonize distant and foreign sites in the body. These metastatic cells survive by overriding restrictions that normally constrain cell colonization into dissimilar tissues. For example, typical mammary epithelial cells will generally not grow or survive if transplanted to the lung, yet lung metastases are a major cause of breast cancer morbidity and mortality. Recent evidence suggests that dissemination of metastatic cells through the body can occur long before clinical presentation of the primary tumor. These micrometastatic cells may remain dormant for many months or years following the detection and removal of the primary tumor. Thus, a better understanding of the mechanisms that allow for the growth and survival of metastatic cells in a foreign microenvironment is critical for the improvement of therapeutics designed to fight metastatic cancer and diagnostics for the early detection and localization of metastases.

2.1.3 Cancer Cell Signaling

[0006] Cancer is a disease of aberrant signal transduction. Aberrant cell signaling overrides anchorage-dependent constraints on cell growth and survival (Rhim *et al.*, 1997, *Crit. Rev. in Oncogenesis* 8:305; Patarca, 1996, *Crit. Rev. in Oncogenesis* 7:343; Malik *et al.*, 1996, *Biochimica et Biophysica Acta* 1287:73; Cance *et al.*, 1995, *Breast Cancer Res. Treat.* 35:105). Tyrosine kinase activity is induced by extracellular matrix (ECM) anchorage and indeed, the expression or function of tyrosine kinases is usually increased in malignant cells (Rhim *et al.*, 1997, *Critical Reviews in Oncogenesis* 8:305; Cance *et al.*, 1995, *Breast Cancer Res. Treat.* 35:105, 1995; Hunter, 1997, *Cell* 88:333). Based on evidence that tyrosine kinase activity is necessary for malignant cell growth, tyrosine kinases have been targeted with new therapeutics (Levitzki *et al.*, 1995, *Science* 267:1782; Kondapaka *et al.*, 1996, *Mol. & Cell. Endocrinol.* 117:53; Fry *et al.*, 1995, *Curr. Opin. in BioTechnology* 6:662). Unfortunately, obstacles associated with specific targeting to tumor cells often limit the application of these drugs. In particular, tyrosine kinase activity is often vital for the function and survival of benign tissues (Levitzki *et al.*, 1995, *Science* 267:1782). To minimize collateral toxicity, it is critical to first identify and then target tyrosine kinases that are selectively overexpressed in tumor cells.

2.1.4 Cancer Therapy

[0007] Barriers to the development of anti-metastasis agents have been the assay systems that are used to design and evaluate these drugs. Most conventional cancer therapies target rapidly growing cells. However, cancer cells do not necessarily grow more rapidly but instead survive and grow under conditions that are non-permissive to normal cells (Lawrence and Steeg, 1996, *World J. Urol.* 14:124-130). These fundamental differences between the behavior of normal and malignant cells provide opportunities for therapeutic targeting. The paradigm that micrometastatic tumors have already disseminated throughout the body emphasizes the need to evaluate potential chemotherapeutic drugs in the context of a foreign and three-dimensional microenvironment. Many standard cancer drug assays measure tumor cell growth or survival under typical cell culture conditions (*i.e.,* monolayer growth). However, cell behavior in two-dimensional assays often does not reliably predict tumor cell behavior *in vivo*.

[0008] Currently, cancer therapy may involve surgery, chemotherapy, hormonal therapy and/or radiation treatment to eradicate neoplastic cells in a patient (*see, e.g.,* Stockdale, 1998, "Principles of Cancer Patient Management," in Scientific American: Medicine, vol. 3, Rubenstein and Federman, eds., ch. 12, sect. IV). Recently, cancer therapy may also involve biological therapy or immunotherapy. All of these approaches can pose significant drawbacks for the patient. Surgery, for example, may be contraindicated due to the health of the patient or may be unacceptable to the patient. Additionally, surgery may not completely remove the neoplastic tissue. Radiation therapy is only effective when the neoplastic tissue exhibits a higher sensitivity to radiation than normal tissue, and radiation therapy can also often elicit serious side effects. Hormonal therapy is rarely given as a single agent and, although it can be effective, is often used to prevent or delay recurrence of cancer after other treatments have removed the majority of the cancer cells. Biological therapies/immunotherapies are limited in number and each therapy is generally effective for only a very specific type of cancer.

[0009] With respect to chemotherapy, there are a variety of chemotherapeutic agents available for treatment of cancer. A significant majority of cancer chemotherapeutics act by inhibiting DNA synthesis, either directly, or indirectly by inhibiting the biosynthesis of the deoxyribonucleotide triphosphate precursors, to prevent DNA replication and concomitant cell division (*see, e.g.*, Gilman *et al.*, 1990, Goodman and Gilman's: The Pharmacological Basis of Therapeutics, 8th Ed. (Pergamom Press, New York)). These agents, which include alkylating agents, such as nitrosourea, anti-metabolites, such as methotrexate and hydroxyurea, and other agents, such as etoposides, campathecins, bleomycin, doxorubicin, daunorubicin, *etc.*, although not necessarily cell cycle specific, kill cells during S phase

because of their effect on DNA replication. Other agents, specifically colchicine and the vinca alkaloids, such as vinblastine and vincristine, interfere with microtubule assembly resulting in mitotic arrest. Chemotherapy protocols generally involve administration of a combination of chemotherapeutic agents to increase the efficacy of treatment.

[0010] Despite the availability of a variety of chemotherapeutic agents, chemotherapy has many drawbacks (*see, e.g.*, Stockdale, 1998, "Principles Of Cancer Patient Management" in Scientific American Medicine, vol. 3, Rubenstein and Federman, eds., ch. 12, sect. X). Almost all chemotherapeutic agents are toxic, and chemotherapy causes significant, and often dangerous, side effects, including severe nausea, bone marrow depression, immunosuppression, *etc.* Additionally, even with administration of combinations of chemotherapeutic agents, many tumor cells are resistant or develop resistance to the chemotherapeutic agents. In fact, those cells resistant to the particular chemotherapeutic agents used in the treatment protocol often prove to be resistant to other drugs, even those agents that act by mechanisms different from the mechanisms of action of the drugs used in the specific treatment; this phenomenon is termed pleiotropic drug or multidrug resistance. Thus, because of drug resistance, many cancers prove refractory to standard chemotherapeutic treatment protocols.

[0011] There is a significant need for alternative cancer treatments, particularly for treatment of cancer that has proved refractory to standard cancer treatments, such as surgery, radiation therapy, chemotherapy, and hormonal therapy. Further, it is uncommon for cancer to be treated by only one method. Thus, there is a need for development of new therapeutic agents for the treatment of cancer and new, more effective, therapy combinations for the treatment of cancer.

2.1.5 OTHER HYPERPROLIFERATIVE DISORDERS

2.1.5.1 Asthma

[0012] Asthma is a disorder characterized by intermittent airway obstruction. In western countries, it affects 15% of the pediatric population and 7.5% of the adult population (Strachan et al., 1994, *Arch. Dis. Child* 70:174-178). Most asthma in children and young adults is initiated by IgE mediated allergy (atopy) to inhaled allergens such as house dust mite and cat dander allergens. However, not all asthmatics are atopic, and most atopic individuals do not have asthma. Thus, factors in addition to atopy are necessary to induce the disorder (Fraser *et al.*, eds.,1994, Synopsis of Diseases of the Chest: 635-53 (WB Saunders Company, Philadelphia); Djukanovic *et al.*, 1990, *Am. Rev. Respir. Dis.* 142:434-457). Asthma is strongly familial, and is due to the interaction between genetic

and environmental factors. The genetic factors are thought to be variants of normal genes ("polymorphisms") which alter their function to predispose to asthma.

[0013] Asthma may be identified by recurrent wheeze and intermittent air flow limitation. An asthmatic tendency may be quantified by the measurement of bronchial hyper-responsiveness in which an individual's dose-response curve to a broncho-constrictor such as histamine or methacholine is constructed. The curve is commonly summarized by the dose which results in a 20% fall in air flow (PD20) or the slope of the curve between the initial air flow measurement and the last dose given (slope).

[0014] In the atopic response, IgE is produced by B-cells in response to allergen stimulation. These antibodies coat mast cells by binding to the high affinity receptor for IgE and initiate a series of cellular events leading to the destabilization of the cell membrane and release of inflammatory mediators. This results in mucosal inflammation, wheezing, coughing, sneezing and nasal blockage.

[0015] Atopy can be diagnosed by (i) a positive skin prick test in response to a common allergen; (ii) detecting the presence of specific serum IgE for allergen; or (iii) by detecting elevation of total serum IgE.

2.5.1.2 COPD

[0016] Chronic obstructive pulmonary disease (COPD) is an umbrella term frequently used to describe two conditions of fixed airways disorders, chronic bronchitis and emphysema. Chronic bronchitis and emphysema are most commonly caused by smoking; approximately 90% of patients with COPD are or were smokers. Although approximately 50% of smokers develop chronic bronchitis, only 15% of smokers develop disabling airflow obstruction. Certain animals, particularly horses, suffer from COPD as well.

[0017] The airflow obstruction associated with COPD is progressive, may be accompanied by airway hyperactivity, and may be partially reversible. Non-specific airway hyper-responsiveness may also play a role in the development of COPD and may be predictive of an accelerated rate of decline in lung function.

[0018] COPD is a significant cause of death and disability. It is currently the fourth leading cause of death in the United States and Europe. Treatment guidelines advocate early detection and implementation of smoking cessation programs to help reduce morbidity and mortality due to the disorder. However, early detection and diagnosis has been difficult for a number of reasons. COPD takes years to develop and acute episodes of bronchitis often are not recognized by the general practitioner as early signs of COPD. Many patients exhibit features of more than one disorder (*e.g.*, chronic bronchitis or asthmatic bronchitis)

making precise diagnosis a challenge, particularly early in the etiology of the disorder. Also, many patients do not seek medical help until they are experiencing more severe symptoms associated with reduced lung function, such as dyspnea, persistent cough, and sputum production. As a consequence, the vast majority of patients are not diagnosed or treated until they are in a more advanced stage of the disorder.

2.1.5.3 Mucin

[0019] Mucins are a family of glycoproteins secreted by the epithelial cells including those at the respiratory, gastrointestinal and female reproductive tracts. Mucins are responsible for the viscoelastic properties of mucus (Thornton *et al.*, 1997, *J. Biol. Chem.* 272:9561-9566). Nine mucin genes are known to be expressed in man: MUC 1, MUC 2, MUC 3, MUC 4, MUC 5AC, MUC 5B, MUC 6, MUC 7 and MUC 8 (Bobek *et al.*, 1993, *J. Biol. Chem.* 268:20563-9; Dusseyn *et al.*, 1997, *J. Biol. Chem.* 272:3168-78; Gendler *et al.*, 1991, *Am. Rev.Resp. Dis.* 144:S42-S47; Gum *et al.*, 1989, *J. Biol. Chem.* 264:6480-6487; Gum *et al.*, 1990, *Biochem. Biophys. Res. Comm.* 171:407-415; Lesuffleur *et al.*, 1995, *J. Biol. Chem.* 270:13665-13673; Meerzaman *et al.*, 1994, *J. Biol. Chem.* 269:12932-12939; Porchet *et al.*, 1991, *Biochem. Biophys. Res. Comm.* 175:414-422; Shankar *et al.*, 1994, *Biochem. J.* 300:295-298; Toribara *et al.*, 1997, *J. Biol. Chem.* 272:16398-403). Many airway disorders such chronic bronchitis, chronic obstructive pulmonary disease, bronchietactis, asthma, cystic fibrosis and bacterial infections are characterized by mucin overproduction (Prescott *et al.*, *Eur. Respir. J.*, 1995, 8:1333-1338; Kim *et al.*, *Eur. Respir. J.*, 1997, 10:1438; Steiger *et al.*, 1995, *Am. J. Respir. Cell Mol. Biol.*, 12:307-314). Mucociliary impairment caused by mucin hypersecretion leads to airway mucus plugging which promotes chronic infection, airflow obstruction and sometimes death. For example, COPD, a disorder characterized by slowly progressive and irreversible airflow limitation, is a major cause of death in developed countries. The respiratory degradation consists mainly of decreased luminal diameters due to airway wall thickening and increased mucus caused by goblet cell hyperplasia and hypersecretion. Epidermal growth factor (EGF) is known to upregulate epithelial cell proliferation, and mucin production/secretion (Takeyama *et al.*, 1999, *Proc. Natl. Acad. Sci. USA* 96:3081-6; Burgel *et al.*, 2001, *J. Immunol.* 167:5948-54). EGF also causes mucin-secreting cells, such as goblet cells, to proliferate and increase mucin production in airway epithelia (Lee *et al.*, 2000, *Am. J. Physiol. Lung Cell. Mol. Physiol.* 278:185-92; Takeyama *et al.*, 2001, *Am. J. Respir. Crit. Care. Med.* 163:511-6; Burgel *et al.*, 2000, *J. Allergy Clin. Immunol.* 106:705-12). Historically, mucus hypersecretion has been treated in two ways: physical methods to increase clearance and mucolytic agents. Neither approach has yielded significant benefit

to the patient or reduced mucus obstruction. Therefore, it would be desirable to have methods for reducing mucin production and treating the disorders associated with mucin hypersecretion.

2.1.5.4 Restenosis

[0020] Vascular interventions, including angioplasty, stenting, atherectomy and grafting are often complicated by undesirable effects. Exposure to a medical device which is implanted or inserted into the body of a patient can cause the body tissue to exhibit adverse physiological reactions. For instance, the insertion or implantation of certain catheters or stents can lead to the formation of emboli or clots in blood vessels. Other adverse reactions to vascular intervention include endothelial cell proliferation which can lead to hyperplasia, restenosis, *i.e.* the re-occlusion of the artery, occlusion of blood vessels, platelet aggregation, and calcification. Treatment of restenosis often involves a second angioplasty or bypass surgery. In particular, restenosis may be due to endothelial cell injury caused by the vascular intervention in treating a restenosis.

[0021] Angioplasty involves insertion of a balloon catheter into an artery at the site of a partially obstructive atherosclerotic lesion. Inflation of the balloon is intended to rupture the intima and dilate the obstruction. About 20 to 30% of obstructions reocclude in just a few days or weeks (Eltchaninoff *et al.*, 1998, *J. Am Coll. Cardiol.* 32: 980-984). Use of stents reduces the re-occlusion rate, however a significant percentage continues to result in restenosis. The rate of restenosis after angioplasty is dependent upon a number of factors including the length of the plaque. Stenosis rates vary from 10% to 35% depending the risk factors present. Further, repeat angiography one year later reveals an apparently normal lumen in only about 30% of vessels having undergone the procedure.

[0022] Restenosis is caused by an accumulation of extracellular matrix containing collagen and proteoglycans in association with smooth muscle cells which is found in both the atheroma and the arterial hyperplastic lesion after balloon injury or clinical angioplasty. Some of the delay in luminal narrowing with respect to smooth muscle cell proliferation may result from the continuing elaboration of matrix materials by neointimal smooth muscle cells. Various mediators may alter matrix synthesis by smooth muscle cells *in vivo*.

2.1.5.5 Neointimal Hyperplasia

[0023] Neointimal hyperplasia is the pathological process that underlies graft atherosclerosis, stenosis, and the majority of vascular graft occlusion. Neointimal hyperplasia is commonly seen after various forms of vascular injury and a major component

of the vein graft's response to harvest and surgical implantation into high-pressure arterial circulation.

[0024] Smooth muscle cells in the middle layer (*i.e.* media layer) of the vessel wall become activated, divide, proliferate and migrate into the inner layer (*i.e.* intima layer). The resulting abnormal neointimal cells express pro-inflammatory molecules, including cytokines, chemokines and adhesion molecules that further trigger a cascade of events that lead to occlusive neointimal disease and eventually graft failure.

[0025] The proliferation of smooth muscle cells is a critical event in the neointimal hyperplastic response. Using a variety of approaches, studies have clearly demonstrated that blockade of smooth muscle cell proliferation resulted in preservation of normal vessel phenotype and function, causing the reduction of neointimal hyperplasia and graft failure.

[0026] Existing treatments for the indications discussed above is inadequate; thus, there exists a need for improved treatments for the above indications.

2.2 EphA2

[0027] EphA2 is a 130 kDa receptor tyrosine kinase that is expressed in adult epithelia, where it is found at low levels and is enriched within sites of cell-cell adhesion (Zantek *et al*, 1999, *Cell Growth & Differentiation* 10:629; Lindberg *et al.*, 1990, *Molecular & Cellular Biology* 10:6316). This subcellular localization is important because EphA2 binds ligands (known as EphrinsA1 to A5) that are anchored to the cell membrane (Eph Nomenclature Committee, 1997, *Cell* 90:403; Gale *et al.*, 1997, *Cell & Tissue Research* 290: 227). The primary consequence of ligand binding is EphA2 autophosphorylation (Lindberg *et al.*, 1990, *supra*). However, unlike other receptor tyrosine kinases, EphA2 retains enzymatic activity in the absence of ligand binding or phosphotyrosine content (Zantek *et al.*, 1999, *supra*). EphA2 is upregulated on a large number hyperproliferating cells, including aggressive carcinoma cells.

3. SUMMARY OF THE INVENTION

[0028] EphA2 is overexpressed and functionally altered in a large number of malignant carcinomas. EphA2 is an oncoprotein and is sufficient to confer metastatic potential to cancer cells. EphA2 is also associated with other hyperproliferating cells and is implicated in diseases caused by cell hyperproliferation. The present invention stems from the inventors' discovery that administration of an expression vehicle for an EphA2 antigenic peptide to a subject provides beneficial therapeutic and prophylactic benefits against hyperproliferative disorders involving EphA2 overexpressing cells. Without being

bound by any mechanism or theory, it is believed that the therapeutic and prophylactic benefit is the result of an immune response elicited against the EphA2 antigenic peptide.

[0029] The present invention thus provides EphA2 vaccines and methods for their use. The EphA2 vaccines of the present invention can elicit or mediate a cellular immune response, a humoral immune response, or both. Where the immune response is a cellular immune response, it can be a Tc, Th1 or a Th2 immune response. In a preferred embodiment, the immune response is a Th2 cellular immune response.

[0030] In a preferred embodiment, an EphA2 vaccine of the invention comprises or encodes one or more epitopes on EphA2 that is selectively exposed or increased on cancer cells relative to not non-cancer cells. In one embodiment, the cancer is of an epithelial cell origin. . In other embodiments, the cancer is a cancer of the skin, lung, colon, prostate, breast, ovary, eosophageal, bladder, or pancreas or is a renal cell carcinoma or a melanoma. In another embodiment, the cancer is of a T cell origin. In yet other embodiments, the cancer is a leukemia or a lymphoma.

[0031] In a preferred embodiment, the methods and compositions of the invention are used to prevent, treat or manage EphA2-expressing tumor metastases. In a preferred embodiment, the EphA2-expressing cells against which an immune response is sought ("target cells") overexpress EphA2. In a preferred embodiment, some EphA2 on the target cells is not bound to ligand, either as a result of decreased cell-cell contacts, altered subcellular localization, or increases in amount of EphA2 relative to ligand.

[0032] Thus, the present invention provides methods of eliciting an immune response against an EphA2-expressing cell, said method comprising administering to an individual an EphA2 vaccine in an amount effective to elicit an immune response against an EphA2-expressing cell.

[0033] The present invention further provides a method of treating or preventing a hyperproliferative disorder of EphA2-expressing cells, said method comprising administering to an individual an EphA2 vaccine in an amount effective treat or prevent the hyperproliferative disorder.

[0034] The present invention yet further provides EphA2 vaccines useful for eliciting an immune response against an EphA2-expressing cell and/or for treating or preventing a hyperproliferative disorder of EphA2-expressing cells.

[0035] The EphA2 vaccines may comprise an EphA2 antigenic peptide, an EphA2 antigenic peptide expression vehicle, an antigen presenting cell sensitized with an EphA2 antigenic peptide, or an anti-idiotypic antibody or antigen-binding fragment thereof which immunospecifically binds to an idiotype of an anti-EphA2 antibody.

[0036] In embodiments where an EphA2 vaccine comprises an EphA2 antigenic peptide, the vaccine may further comprise an adjuvant, or a heat shock protein bound to the EphA2 antigenic peptide.

[0037] In certain embodiments, the EphA2 antigenic peptide comprises a protein transduction domain, for example the protein transduction domain is the Antennapedia or the HIV tat protein transduction domain.

[0038] In certain embodiments in which an EphA2 vaccine comprises an EphA2 antigenic peptide expression vehicle, the expression vehicle can be a nucleic acid, preferably DNA, encoding said EphA2 antigenic polypeptide operably linked to a promoter. The DNA can be conjugated to a carrier, including but not limited to an asialoglycoprotein carrier, a transferrin carrier, or a polymeric IgA carriers.

[0039] In other embodiments, the expression vehicle is an infectious agent comprising a nucleic acid, said nucleic acid comprising a nucleotide sequence encoding said EphA2 antigenic polypeptide operably linked to a promoter. Preferably, the sequence encoding said EphA2 antigenic polypeptide is codon-optimized for expression in said infectious agent and/or the infectious agent is coated with a reagent that targets the infectious agent to EphA2-expressing cells (such as an EphA2 antibody) or to antigen-presenting cells.

[0040] A preferred infectious agent for use as an EphA2 antigenic peptide expression vehicle in accordance with the methods and compositions of the invention is a bacterium. Preferred bacteria for administration to human subjects are attenuated, for example are deficient in DNA repair (*e.g.*, mutant in a DNA repair gene) or subjected to psoralen-treatment. Preferably, the nucleic acid encoding the EphA2 antigenic peptide comprises a nucleotide sequence encoding a secretory signal, *e.g.*, the SecA secretory signal, operatively linked to the sequence encoding the EphA2 antigenic polypeptide. A preferred strain of bacteria for use in the methods and compositions of the invention is *Pseudomonas aeruginosa*. In certain specific embodiments, the bacteria is not *Listeria*, and more preferably is not *Listeria monocytogenes*.

[0041] Another preferred infectious agent for use as an EphA2 antigenic peptide expression vehicle in accordance with the methods and compositions of the invention is a virus, for example a retrovirus, including but not limited to lentivirus, an adenovirus, an adeno-associated virus, or a herpes simplex virus. Preferred viruses for administration to human subjects are attenuated viruses.

[0042] As an alternative to an infectious agent or nucleic acid, an EphA2 antigenic peptide expression vehicle can be a mammalian cell comprising a recombinant nucleic acid,

said nucleic acid comprising a nucleotide sequence encoding said EphA2 antigenic polypeptide. Preferably, the mammalian cell is a human cell. Mammalian cells for use in the methods and compositions of the invention may be encapsulated within a membrane, for example a THERACYTE membrane, and/or administered by means of implantation.

[0043] Compositions of the present invention useful as EphA2 vaccines also include anti-idiotypes of anti-EphA2 antibodies. In certain specific embodiments, the EphA2 vaccines comprise anti-idiotypes of the anti-EphA2 monoclonal antibodies secreted by the hybridoma clones deposited in the ATCC as PTA-4572, PTA-4573, and PTA-4574.

[0044] With respect to EphA2 vaccines comprising sensitized antigen presenting cells, such as macrophages and dendritic cells, in certain embodiments, the antigen presenting cells are sensitized prior to their administration. For example, the antigen presenting cells may be sensitized by a method comprising: (a) contacting the cells with a composition comprising one or more EphA2 antigenic peptides, and optionally comprising one or more heat shock proteins such as hsp70, gp96, or hsp90, in an amount effective to sensitize the cells. In a preferred embodiment, the antigen presenting cells are autologous to the individual to whom they are administered; however, the cells need not be autologous.

[0045] The compositions and methods of the present invention are useful in the treatment of hyperproliferative diseases. In certain embodiments, the hyperproliferative disease is cancer. In certain embodiments, the cancer is of an epithelial cell origin and/or involves cells that overexpress EphA2 relative to non-cancer cells having the tissue type of said cancer cells. In specific embodiments, the cancer is a cancer of the skin, lung, colon, breast, ovarian, esophogeal, prostate, bladder or pancreas or is a renal cell carcinoma or melanoma. In yet other embodiments, the cancer is of a T cell origin. In specific embodiments, the cancer is a leukemia or a lymphoma. In yet other embodiments, the hyperproliferative disorder is non-neoplastic. In specific embodiments, the non-neoplastic hyperproliferative disorder is an epithelial cell disorder. Exemplary non-neoplastic hyperproliferative disorders are asthma, chronic pulmonary obstructive disease, lung fibrosis, bronchial hyper responsiveness, psoriasis, and seborrheic dermatitis.

[0046] The EphA2 antigenic polypeptide for use in accordance with the methods and compositions of the present invention may comprise full length EphA2 or an antigenic fragment or derivative thereof. In certain embodiments, the EphA2 antigenic polypeptide comprises the extracellular domain of EphA2 or the intracellular domain of EphA2. In certain embodiments the EphA2 antigenic polypeptide is a chimeric polypeptide comprising at least an antigenic portion of EphA2 and a second polypeptide.

[0047] A vaccine of the invention may have one or a plurality of EphA2 antigenic polypeptides, a plurality of EphA2 antigenic polypeptide expression vehicles, or antigen presenting cells sensitized with a plurality of EphA2 antigenic polypeptides. A vaccine of the invention may also have one or more classes of immune response-inducing or -mediating reagents, for example both an EphA2 antigenic polypeptide and an EphA2 antigenic polypeptide expression vehicle, both an EphA2 antigenic polypeptide and an antigen-presenting cell sensitized with an EphA2 antigenic polypeptide, or both an EphA2 antigenic polypeptide expression vehicle and an antigen-presenting cell sensitized with an EphA2 antigenic polypeptide.

[0048] The methods of the present invention encompass combination therapy with an EphA2 vaccine and one or more additional therapeutics, for example an additional anti-cancer therapy. In certain embodiments, the additional anti-cancer therapy is an agonistic EphA2 antibody. In other embodiments, the additional anti-cancer therapy is chemotherapy, biological therapy, immunotherapy, radiation therapy, hormonal therapy, or surgery.

[0049] The vaccines of the invention can be administered by mucosal, intranasal, parenteral, intramuscular, or intraperitoneal routes.

[0050] In other embodiments, the EphA2 vaccines of the invention are used to treat, prevent and/or manage a non-cancer disease or disorder associated with cell hyperproliferation, such as but not limited to asthma, chronic obstructive pulmonary disease, restenosis (smooth muscle and/or endothelial), psoriasis, etc. In preferred embodiments, the hyperproliferative cells are epithelial. In preferred embodiments, the hyperproliferative cells overexpress EphA2. In a preferred embodiment, some EphA2 is not bound to ligand, either as a result of decreased cell-cell contacts, altered subcellular localization, or increases in amount of EphA2 relative to EphA2-ligand.

[0051] The methods and compositions of the invention are useful not only in untreated patients but are also useful in the treatment of patients partially or completely refractory to current standard and experimental cancer therapies, including but not limited to chemotherapies, hormonal therapies, biological therapies, radiation therapies, and/or surgery as well as to improve the efficacy of such treatments. In particular, EphA2 expression has been implicated in increasing levels of the cytokine IL-6, which has been associated with the development of cancer cell resistance to different treatment regimens, such as chemotherapy and hormonal therapy. In addition, EphA2 overexpression can override the need for estrogen receptor activity thus contributing to tamoxifen resistance in breast cancer cells. Accordingly, in a preferred embodiment, the invention provides

therapeutic and prophylactic methods for the treatment or prevention of cancer that has been shown to be or may be refractory or non-responsive to therapies other than those comprising administration of EphA2 antibodies of the invention. In a specific embodiment, one or more EphA2 vaccines of the invention are administered to a patient refractory or non-responsive to a non-EphA2-based treatment, particularly tamoxifen treatment or a treatment in which resistance is associated with increased IL-6 levels, to render the patient non-refractory or responsive. The treatment to which the patient had previously been refractory or non-responsive can then be administered with therapeutic effect.

[0052] In another embodiment, kits comprising the vaccines or vaccine components of the invention are provided.

3.1 DEFINITIONS

[0053] As used herein, the term "EphA2 vaccine" can be any reagent that elicits or mediates an immune response against EphA2 on hyperproliferative cells. In certain embodiments, an EphA2 vaccine is an EphA2 antigenic peptide of the invention, an expression vehicle (*e.g.*, a naked nucleic acid or a viral or bacterial vector or a cell) for an EphA2 antigenic peptide, or T cells or antigen presenting cells (*e.g.*, dendritic cells or macrophages) that have been primed with the EphA2 antigenic peptide of the invention.

[0054] As used herein, the term "EphA2 antigenic peptide" or "EphA2 antigenic polypeptide" refers to an EphA2 polypeptide, preferably of SEQ ID NO:2, or a fragment or derivative thereof comprising one or more B cell epitopes or T cell epitopes of EphA2. In certain embodiments, the EphA2 antigenic peptides are not one or more of the following peptides: TLADFDPRV (SEQ ID NO:3); VLLLVLAGV (SEQ ID NO:4); VLAGVGFFI (SEQ ID NO:5); IMNDMPIYM (SEQ ID NO:6); SLLGLKDQV (SEQ ID NO:7); WLVPIGQCL (SEQ ID NO:8); LLWGCALAA (SEQ ID NO:9); GLTRTSVTV (SEQ ID NO:10); NLYYAESDL (SEQ ID NO:11); KLNVEERSV (SEQ ID NO:12); IMGQFSHHN (SEQ ID NO:13); YSVCNVMSG (SEQ ID NO:14); MQNIMNDMP (SEQ ID NO:15); EAGIMGQFSHHNIIR (SEQ ID NO:16); PIYMYSVCNVMSG (SEQ ID NO:17); DLMQNIMNDMPIYMYS (SEQ ID NO:18). In certain specific embodiments, the EphA2 antigenic peptide is not any of SEQ ID NO:3-12, is not SEQ ID NO:13-15, and/or is not SEQ ID NO:16-18. In yet another specific enbodiment, the EphA2 antigenic peptide is not SEQ ID NO:3-18.

[0055] The term "derivative" as used herein refers to a polypeptide that comprises an amino acid sequence of an EphA2 polypeptide or a fragment of an EphA2 polypeptide that has been altered by the introduction of amino acid residue substitutions, deletions or additions (*i.e.*, mutations). The term "derivative" as used herein also refers to an EphA2

polypeptide or a fragment of an EphA2 polypeptide which has been modified, *i.e*, by the covalent attachment of any type of molecule to the polypeptide. For example, but not by way of limitation, an EphA2 polypeptide or a fragment of an EphA2 polypeptide may be modified, *e.g.*, by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, *etc.* A derivative of an EphA2 polypeptide or a fragment of an EphA2 polypeptide may be modified by chemical modifications using techniques known to those of skill in the art, including, but not limited to, specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, *etc.* Further, a derivative of an EphA2 polypeptide or a fragment of an EphA2 polypeptide may contain one or more non-classical amino acids. In one embodiment, a polypeptide derivative possesses a similar or identical function as an EphA2 polypeptide or a fragment of an EphA2 polypeptide described herein. In another embodiment, a derivative of EphA2 polypeptide or a fragment of an EphA2 polypeptide has an altered activity when compared to an unaltered polypeptide. For example, a derivative of an EphA2 polypeptide or fragment thereof can differ in phosphorylation relative to an EphA2 polypeptide or fragment thereof.

[0056] The term "B cell epitope" as used herein refers to a portion of an EphA2 polypeptide having antigenic or immunogenic activity in an animal, preferably in a mammal, and most preferably in a mouse or a human. An epitope having immunogenic activity is a portion of an EphA2 polypeptide that elicits an antibody response in an animal. An epitope having antigenic activity is a portion of an EphA2 polypeptide to which an antibody immunospecifically binds as determined by any method well known in the art, for example, by immunoassays. Antigenic epitopes need not necessarily be immunogenic.

[0057] The term "T cell epitope" as used herein refers to at least a portion of an EphA2 polypeptide, preferably an EphA2 polypeptide of SEQ ID NO:2, that is recognized by a T cell receptor. The term "T cell epitope" encompasses helper T cell (Th) epitopes and cytotoxic T cell (Tc) epitopes. The term "helper T cell epitopes" encompasses Th1 and Th2 epitopes.

[0058] The "fragments" described herein include an EphA2 antigenic peptide or polypeptide comprising an amino acid sequence of at least 5 contiguous amino acid residues, at least 10 contiguous amino acid residues, at least 15 contiguous amino acid residues, at least 20 contiguous amino acid residues, at least 25 contiguous amino acid residues, at least 40 contiguous amino acid residues, at least 50 contiguous amino acid residues, at least 60 contiguous amino residues, at least 70 contiguous amino acid residues, at least 80 contiguous amino acid residues, at least 90 contiguous amino acid residues, at

least 100 contiguous amino acid residues, at least 125 contiguous amino acid residues, at least 150 contiguous amino acid residues, at least 175 contiguous amino acid residues, at least 200 contiguous amino acid residues, or at least 250 contiguous amino acid residues of the amino acid sequence of an EphA2 polypeptide.

[0059] As used herein, the term "in combination" refers to the use of more than one prophylactic and/or therapeutic agents. The use of the term "in combination" does not restrict the order in which prophylactic and/or therapeutic agents are administered to a subject with a hyperproliferative cell disorder, especially cancer. A first prophylactic or therapeutic agent can be administered prior to (*e.g.*, 1 minute, 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks before), concomitantly with, or subsequent to (*e.g.*, 1 minute, 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks after) the administration of a second prophylactic or therapeutic agent to a subject which had, has, or is susceptible to a hyperproliferative cell disorder, especially cancer. The prophylactic or therapeutic agents are administered to a subject in a sequence and within a time interval such that the agent of the invention can act together with the other agent to provide an increased benefit than if they were administered otherwise. Any additional prophylactic or therapeutic agent can be administered in any order with the other additional prophylactic or therapeutic agents.

[0060] As used herein, the phrase "low tolerance" refers to a state in which the patient suffers from side effects from treatment so that the patient does not benefit from and/or will not continue therapy because of the adverse effects and/or the harm from the side effects outweighs the benefit of the treatment.

[0061] As used herein, the terms "manage," "managing" and "management" refer to the beneficial effects that a subject derives from administration of a prophylactic or therapeutic agent, which does not result in a cure of the disease. In certain embodiments, a subject is administered one or more prophylactic or therapeutic agents to "manage" a disease so as to prevent the progression or worsening of the disease.

[0062] As used herein, the phrase "non-responsive/refractory" is used to describe patients treated with one or more currently available therapies (*e.g.*, cancer therapies) such as chemotherapy, radiation therapy, surgery, hormonal therapy and/or biological therapy/immunotherapy, particularly a standard therapeutic regimen for the particular cancer, wherein the therapy is not clinically adequate to treat the patients such that these

patients need additional effective therapy, *e.g.*, remain unsusceptible to therapy. The phrase can also describe patients who respond to therapy yet suffer from side effects, relapse, develop resistance, etc. In various embodiments, "non-responsive/refractory" means that at least some significant portion of the cancer cells are not killed or their cell division arrested. The determination of whether the cancer cells are "non-responsive/refractory" can be made either *in vivo* or *in vitro* by any method known in the art for assaying the effectiveness of treatment on cancer cells, using the art-accepted meanings of "refractory" in such a context. In various embodiments, a cancer is "non-responsive/refractory" where the number of cancer cells has not been significantly reduced, or has increased during the treatment.

[0063] As used herein, the term "potentiate" refers to an improvement in the efficacy of a therapeutic agent at its common or approved dose.

[0064] As used herein, the terms "prevent," " preventing" and "prevention" refer to the prevention of the onset, recurrence, or spread of a disease in a subject resulting from the administration of a prophylactic or therapeutic agent.

[0065] As used herein, the term "prophylactic agent" refers to any agent that can be used in the prevention of the onset, recurrence or spread of a disease or disorder associated with EphA2 overexpression and/or cell hyperproliferative disease, particularly cancer. In certain embodiments, the term "prophylactic agent" refers to an EphA2 vaccine of the invention, such as a composition comprising an EphA2 antigenic peptide, an EphA2 antigenic peptide expression vehicle, or an antigen presenting cell sensitized with an EphA2 antigenic peptide. In certain other embodiments, the term "prophylactic agent" refers to cancer chemotherapeutics, radiation therapy, hormonal therapy, biological therapy (*e.g.*, immunotherapy). In other embodiments, more than one prophylactic agent may be administered in combination.

[0066] As used herein, a "prophylactically effective amount" refers to that amount of the prophylactic agent sufficient to result in the prevention of the onset, recurrence or spread of cell hyperproliferative disease, preferably, cancer. A prophylactically effective amount may refer to the amount of prophylactic agent sufficient to prevent the onset, recurrence or spread of hyperproliferative disease, particularly cancer, including but not limited to those predisposed to hyperproliferative disease, for example, those genetically predisposed to cancer or previously exposed to carcinogens. A prophylactically effective amount may also refer to the amount of the prophylactic agent that provides a prophylactic benefit in the prevention of hyperproliferative disease. Further, a prophylactically effective amount with respect to a prophylactic agent of the invention means that amount of prophylactic agent alone, or in combination with other agents, that provides a prophylactic

benefit in the prevention of hyperproliferative disease. Used in connection with an amount of an EphA2 vaccine of the invention, the term can encompass an amount that improves overall prophylaxis or enhances the prophylactic efficacy of or synergies with another prophylactic agent.

[0067] As used herein, a "protocol" includes dosing schedules and dosing regimens.

[0068] As used herein, the phrase "side effects" encompasses unwanted and adverse effects of a prophylactic or therapeutic agent. Adverse effects are always unwanted, but unwanted effects are not necessarily adverse. An adverse effect from a prophylactic or therapeutic agent might be harmful or uncomfortable or risky. Side effects from chemotherapy include, but are not limited to, gastrointestinal toxicity such as, but not limited to, early and late-forming diarrhea and flatulence, nausea, vomiting, anorexia, leukopenia, anemia, neutropenia, asthenia, abdominal cramping, fever, pain, loss of body weight, dehydration, alopecia, dyspnea, insomnia, dizziness, mucositis, xerostomia, and kidney failure, as well as constipation, nerve and muscle effects, temporary or permanent damage to kidneys and bladder, flu-like symptoms, fluid retention, and temporary or permanent infertility. Side effects from radiation therapy include but are not limited to fatigue, dry mouth, and loss of appetite. Side effects from biological therapies/immunotherapies include but are not limited to rashes or swellings at the site of administration, flu-like symptoms such as fever, chills and fatigue, digestive tract problems and allergic reactions. Side effects from hormonal therapies include but are not limited to nausea, fertility problems, depression, loss of appetite, eye problems, headache, and weight fluctuation. Additional undesired effects typically experienced by patients are numerous and known in the art. Many are described in the *Physicians' Desk Reference* (56^{th} ed., 2002).

[0069] As used herein, the terms "subject" and "patient" are used interchangeably. As used herein, a subject is preferably a mammal such as a non-primate (*e.g.*, cows, pigs, horses, cats, dogs, rats *etc.*) and a primate (*e.g.*, monkey and human), most preferably a human.

[0070] As used herein, the terms "treat," "treating" and "treatment" refer to the eradication, reduction or amelioration of symptoms of a disease or disorder, particularly, the eradication, removal, modification, or control of primary, regional, or metastatic cancer tissue that results from the administration of one or more therapeutic agents. In certain embodiments, such terms refer to the minimizing or delaying the spread of cancer resulting from the administration of one or more therapeutic agents to a subject with such a disease.

[0071] As used herein, the term "therapeutic agent" refers to any agent that can be used in the prevention, treatment, or management of a disease or disorder associated with overexpression of EphA2 and/or cell hyperproliferative diseases or disorders, particularly, cancer. In certain embodiments, the term "therapeutic agent" refers to an EphA2 vaccine of the invention, such as a composition comprising an EphA2 antigenic peptide, an EphA2 antigenic peptide expression vehicle, or an antigen presenting cell sensitized with an EphA2 antigenic peptide. In certain other embodiments, the term "therapeutic agent" refers to cancer chemotherapeutics, radiation therapy, hormonal therapy, and/or biological therapy/immunotherapy. In other embodiments, more than one therapeutic agent may be administered in combination.

[0072] As used herein, a "therapeutically effective amount" refers to that amount of the therapeutic agent sufficient to treat or manage a disease or disorder associated with EphA2 overexpression and/or cell hyperproliferative disease and, preferably, the amount sufficient to destroy, modify, control or remove primary, regional or metastatic cancer tissue. A therapeutically effective amount may refer to the amount of therapeutic agent sufficient to delay or minimize the onset of the hyperproliferative disease, *e.g.*, delay or minimize the spread of cancer. A therapeutically effective amount may also refer to the amount of the therapeutic agent that provides a therapeutic benefit in the treatment or management of cancer. Further, a therapeutically effective amount with respect to a therapeutic agent of the invention means that amount of therapeutic agent alone, or in combination with other therapies, that provides a therapeutic benefit in the treatment or management of hyperproliferative disease or cancer. Used in connection with an amount of an EphA2 vaccine of the invention, the term can encompass an amount that improves overall therapy, reduces or avoids unwanted effects, or enhances the therapeutic efficacy of or synergies with another therapeutic agent.

[0073] As used herein, the terms "T cell malignancies" and "T cell malignancy" refer to any T cell lymphoproliferative disorder, including thymic and post-thymic malignancies. T cell malignancies include tumors of T cell origin. T cell malignancies refer to tumors of lymphoid progenitor cell, thymocyte, T cell, NK-cell, or antigen presenting cell origin. T cell malignancies include, but are not limited to, leukemias, including acute lymphoblastic leukemias, thymomas, acute lymphoblastic leukemias, and lymphomas, including Hodgkin's and non-Hodgkin's disease, with the proviso that T cell malignancies are not cutaneous T cell malignancies, in particular cutaneous-cell lymphomas. In a preferred embodiment, T cell malignancies are systemic, non-cutaneous T cell malignancies.

3.2 SEQUENCES

[0074] Below is a brief summary of the sequence presented in the accompanying sequence listing, which is incorporated by reference herein in its entirety:

[0075] **SEQ ID NO:1**

Human EphA2 cDNA (full length)

[0076] **SEQ ID NO:2**

Human EphA2 polypeptide (full length)

[0077] **SEQ ID NOs:3-18**

Human EphA2 peptides

[0078] **SEQ ID NO:19**

Construct: LLOss-PEST-hEphA2

Native LLO signal peptide + PEST fused to full-length human EphA2

Not Codon optimized

No epitope tags (e.g., myc or FLAG used in this construct)

Fusion protein coding sequence shown

[0079] **SEQ ID NO:20**

Construct: LLOss-PEST-hEphA2

Native LLO signal peptide + PEST fused to full-length human EphA2

Not Codon optimized

No epitope tags (e.g., myc or FLAG used in this construct)

Predicted fusion protein shown

[0080] **SEQ ID NO:21**

EphA2 EX2 domain

Native nucleotide sequence

[0081] **SEQ ID NO:22**

EphA2 EX2 domain

Nucleotide sequence for optimal codon usage in Listeria

[0082] **SEQ ID NO:23**

EphA2 EX2 domain

Primary Amino Acid Sequence

[0083] **SEQ ID NO:24**

Construct: LLOss-PEST-EX2_hEphA2

Native LLO signal peptide + PEST fused to external domain of human EphA2

Not Codon optimized

No epitope tags (e.g., myc or FLAG used in this construct)

[0084] **SEQ ID NO:25**

Construct: LLOss-PEST-EX2_hEphA2

Native LLO signal peptide + PEST fused to external domain of human EphA2

Not Codon optimized

No epitope tags (e.g., myc or FLAG used in this construct)

Predicted fusion protein shown

[0085] **SEQ ID NO:26**

NativeLLOss-PEST-FLAG-EX2_EphA2-myc-CodonOp

(Native L. monocytogenes LLO signal peptide + PEST-Codon optimized - FLAG-EX-2 EphA2-Myc)

Nucleotide Sequence (including *hly* promoter)

[0086] **SEQ ID NO:27**

NativeLLOss-PEST-FLAG-EX2_EphA2-myc-CodonOp

(Native L. monocytogenes LLO signal peptide + PEST-Codon optimized - FLAG-EX-2 EphA2-Myc)

Primary Amino Acid Sequence

[0087] **SEQ ID NO:28**

Codon Optimized LLOss-PEST-FLAG-EX2_EphA2-myc-CodonOp

(Codon Optimized L. monocytogenes LLO signal peptide + PEST-Codon optimized -FLAG-EX-2 EphA2-Myc)

Nucleotide Sequence (including *hly* promoter)

[0088] **SEQ ID NO:29**

Codon Optimized LLOss-PEST-FLAG-EX2_EphA2-myc-CodonOp

(Codon Optimized L. monocytogenes LLO signal peptide + PEST-Codon optimized -FLAG-EX-2 EphA2-Myc)

Primary Amino Acid Sequence

[0089] **SEQ ID NO:30**

PhoD-FLAG-EX2_EphA2-myc-CodonOp

(Codon optimized B. subtilis phoD Tat signal peptide-FLAG-EX-2 EphA2-Myc)

Nucleotide Sequence (including *hly* promoter)

[0090] **SEQ ID NO:31**

PhoD-FLAG-EX2_EphA2-myc-CodonOp

(Codon optimized B. subtilis phoD Tat signal peptide-FLAG-EX-2 EphA2-Myc)

Amino acid sequence

[0091] **SEQ ID NO:32**

EphA2 CO domain

Native nucleotide sequence

[0092] **SEQ ID NO:33**

EphA2 CO domain

Nucleotide sequence for optimal codon usage in Listeria

[0093] **SEQ ID NO:34**

EphA2 CO domain

Primary Amino Acid Sequence

[0094] **SEQ ID NO:35**

Construct: LLOss-PEST-CO-huEphA2

Native LLO signal peptide + PEST fused to cytoplasmic domain of human EphA2

Not Codon optimized

No epitope tags (e.g., myc or FLAG used in this construct)

Fusion protein coding sequence shown

[0095] **SEQ ID NO:36**

Construct: LLOss-PEST-CO-huEphA2

Native LLO signal peptide + PEST fused to cytoplasmic domain of human EphA2

Not Codon optimized

No epitope tags (e.g., myc or FLAG used in this construct)

Predicted fusion protein shown

[0096] **SEQ ID NO:37**

NativeLLOss-PEST-FLAG-CO_EphA2-myc-CodonOp

(Native L. monocytogenes LLO signal peptide + PEST-Codon optimized - FLAG-CO_EphA2-Myc)

Nucleotide Sequence (including *hly* promoter)

[0097] **SEQ ID NO:38**

NativeLLOss-PEST-FLAG-CO_EphA2-myc-CodonOp

(Native L. monocytogenes LLO signal peptide + PEST-Codon optimized - FLAG-CO_EphA2-Myc)

Primary Amino Acid Sequence

[0098] **SEQ ID NO:39**

Codon Optimized LLOss-PEST-FLAG-CO_EphA2-myc-CodonOp

(Codon Optimized L. monocytogenes LLO signal peptide + PEST-Codon optimized -FLAG-CO_EphA2-Myc)

Nucleotide Sequence (including *hly* promoter)

[0099] **SEQ ID NO:40**

Codon Optimized LLOss-PEST-FLAG-CO_EphA2-myc-CodonOp

(Codon Optimized L. monocytogenes LLO signal peptide + PEST-Codon optimized -FLAG-CO_EphA2-Myc)

Primary Amino Acid Sequence

[00100] **SEQ ID NO:41**

PhoD-FLAG-CO_EphA2-myc-CodonOp

(Codon optimized B. subtilis phoD Tat signal peptide-FLAG-CO_EphA2-Myc)

Nucleotide Sequence (including *hly* promoter)

[00101] **SEQ ID NO:42**

PhoD-FLAG-CO_EphA2-myc-CodonOp

(Codon optimized B. subtilis phoD Tat signal peptide-FLAG-CO_EphA2-Myc)

Amino acid sequence

[00102] **SEQ ID NO:43**

Construct: pAM401-MCS

Plasmid pAM401 containing multiple cloning site (MCS) from pPL2 vector

Insertion of small *Aat II* MCS fragment from pPL2 inserted into pAM401 plasmid between blunted *Xba I* and *Nru I* sites.

Complete pAM401-MCS plasmid sequence shown

4. BRIEF DESCRIPTION OF THE FIGURES

[00103] **Figure 1.** Western blot analysis of secreted protein from recombinant *Listeria* encoding native EphA2 CO domain sequence.

[00104] **Figure 2.** Western blot analysis of secreted protein from recombinant *Listeria* encoding native or codon-optimized LLO secA1 signal peptide fused with codon-optimized EphA2 EX2 domain sequence signal peptide.

[00105] **Figure 3.** Western blot analysis of secreted protein from recombinant *Listeria* encoding native or codon-optimized LLO secA2 signal peptide or codon-optimized Tat signal peptide fused with codon-optimized EphA2 CO domain sequence.

[00106] **Figure 4.** Flow cytometry analysis of human EphA2 expression in CT2 murine carcinoma cells. Single cell FACS sorting assays were performed by standard techniques to identify CT26 cell clones expressing high levels of human EphA2.

[00107] **Figure 5.** Western blot analysis of pooled populations CT26 murine colon carcinoma cells expressing high levels of human EphA2 protein.

[00108] **Figure 6.** Flow Cytometry of B16F10 cells expressing huEphA2.

[00109] **Figure 7.** Western blot analysis of lysate from 293 cells 48 hr. following transfection with pCDNA4 plasmid DNA encoding full-length native EphA2 sequence.

[00110] **Figure 8A-8C.** Figures 8A-8C illustrate results of a typical therapeutic study of animals inoculated with CT26 murine colon carcinoma cells transfected with human EphA2 (L4029-EphA2 exFlag), *Listeria* control (L4029-control) or vehicle (HBSS). In **Figure 8A**, tumor volume was measured at several intervals post inoculation. **Figure 8B** illustrates the mean tumor volume of mice inoculated with CT26 cells containing either *Listeria* control or the ECD of huEphA2. **Figure 8C** represents the results of a therapeutic study using the lung metastases model, measuring percent survival of mice post inoculation with CT26 cells with either HBSS or *Listeria control*, or *Listeria* expressing the ECD of huEphA2.

[00111] **Figures 9A-9D.** Preventive studies following i.v. administration of L4029EphA2-exFlag, *Listeria* control (L4029), or *Listeria* positive control containing the AH1 protein (L4029-AH1) ($5x10^5$ cells in 100 µl volume) either subcutaneously or intravenously. **Figure 9A** demonstrates tumor volume of mice inoculated with CT26 cells expressing the ECD of huEphA2, vehicle (HBSS), *Listeria* (L4029) or *Listeria* positive (L4029-AH1) controls. **Figure 9B** demonstrates mean tumor volume of mice inoculated with CT26 cells expressing the ECD of huEphA2 (L4029-EphA2 exFlag) compared to the *Listeria* (L4029) control. **Figure 9C** illustrates results of the prevention study in the s.c. model, measuring percent survival of the mice post CT26 tumor cell inoculation. **Figure 9D** illustrates the results of the prevention study in the lung metastases model, measuring percent survival of the mice post tumor cell inoculation.

[00112] **Figure 10.** Therapeutic efficacy in Balb/C mice bearing CT26 tumors encoding human EphA2 immunized with recombinant *Listeria* encoding codon-optimized EphA2.

[00113] **Figure 11.** Increased survival of Balb/C mice bearing CT26.24 (huEphA2+) lung tumors when immunized with recombinant *Listeria* encoding codon-optimized secA1 signal peptide fused with condon-optimized EphA2 EX2 domain sequence.

[00114] **Figure 12.** Immunization of Balb/C mice bearing CT26.24 (huEphA2+) lung tumors with recombinant *Listeria* encoding EphA2 CO domain confers long-term survival.

[00115] **Figure 13.** Immunization of Balb/C mice bearing CT26.24 (huEphA2+) lung tumors with recombinant *Listeria* encoding EphA2 CO domain but not with plasmid DNA encoding full-length EphA2 confers long-term survival.

5. DETAILED DESCRIPTION OF THE INVENTION

[00116] The present invention is based, in part, on the inventors' discovery that a vaccine that comprises an EphA2 antigenic peptide can confer beneficial immune response against EphA2-expressing cells in involved in a hyperproliferative disease such as cancer. In particular, such a vaccine can contain an EphA2 antigenic peptide, an expression vehicle for an EphA2 antigenic peptide, or an antigen-presenting cell that is sensitized with an EphA2 antigenic peptide.

[00117] Accordingly, the present invention relates to methods and compositions that provide for the treatment, inhibition, and management of diseases and disorders associated with overexpression of EphA2 and/or cell hyperproliferative diseases and disorders. A particular aspect of the invention relates to methods and compositions containing compounds that, when administered to a subject with a hyperproliferative disorder involving EphA2-expressing cells, either elicit or mediate an immune response against EphA2, resulting in a growth inhibition of the EphA2-expressing cells involved in the hyperproliferative disorder. The present invention further relates to methods and compositions for the treatment, inhibition, or management of metastases of cancers of epithelial cell origin, especially human cancers of the breast, ovary, oesophagus, lung, skin, prostate, bladder, and pancreas, and renal cell carcinomas and melanomas. The invention further relates to methods and compositions for the treatment, inhibition, or management of cancers of T cell origin, especially leukemias and lymphomas. Further compositions and methods of the invention include other types of active ingredients in combination with the EphA2 vaccines of the invention. In other embodiments, the methods of the invention are used to treat, prevent or manage other diseases or disorders associated with cell hyperproliferation, for example but not limited to asthma, psoriasis, restenosis, COPD, *etc.*

[00118] The present invention also relates to methods for the treatment, inhibition, and management of cancer or other hyperproliferative cell disorders or diseases that have

become partially or completely refractory to current or standard cancer treatment, such as chemotherapy, radiation therapy, hormonal therapy, and biological-/immuno-therapy.

5.1 EphA2 Antigenic Peptides

[00119] As discussed above, the invention encompasses administration of exogenous EphA2 antigenic peptides that are capable of eliciting an immune response to EphA2, resulting in a cellular or humoral immune response against endogenous EphA2. Additionally, the present invention encompasses the use of an EphA2 antigenic peptide expression vehicle.

[00120] In principle, an EphA2 antigenic peptide (sometimes referred to as an "EphA2 antigenic polypeptide") for use in the methods and compositions of the present invention can be any EphA2 antigenic peptide that is capable of eliciting an immune response against EphA2-expressing cells involved in a hyperproliferative disorder. Thus, an EphA2 antigenic peptide can be an EphA2 polypeptide, preferably an EphA2 polypeptide of SEQ ID NO:2, or a fragment or derivative of an EphA2 polypeptide that (1) displays antigenicity of EphA2 (ability to bind or compete with EphA2 for binding to an anti-EphA2 antibody, (2) displays immunogenicity of EphA2 (ability to generate antibody which binds to EphA2), or (3) contains one or more T cell epitopes of EphA2.

[00121] In certain embodiments, the EphA2 antigenic peptide is full length human EphA2 (SEQ ID NO:2).

[00122] In other embodiments, the EphA2 antigenic peptide comprises the intracellular domain of EphA2 (residue 22 to 554 of SEQ ID NO:2).

[00123] In yet other embodiments, the EphA2 antigenic peptide comprises the intracellular domain EphA2 (residue 558 to 976 of SEQ ID NO:2).

[00124] In certain embodiments, the peptide corresponds to or comprises an EphA2 epitope that is exposed in a cancer cell but occluded in a non-cancer cell. In a preferred embodiment, the EphA2 antigenic peptides preferentially include epitopes on EphA2 that are selectively exposed or increased on cancer cells but not non-cancer cells ("exposed EphA2 epitope peptides").

[00125] The present invention further encompasses the use of a plurality of EphA2 antigenic peptides in the compositions and methods of the present invention. In certain embodiments, the plurality of EphA2 antigenic peptides are multimerized or polyvalent.

[00126] Fragments of EphA2 that are useful in the methods and compositions present invention may contain deletions, additions or substitutions of amino acid residues within the amino acid sequence encoded by an EphA2 gene. Preferably mutations result in a silent change, thus producing a functionally equivalent EphA2 gene product.

[00127] An EphA2 antigenic polypeptide sequence preferably comprises an amino acid sequence that exhibits at least about 65% sequence similarity to human EphA2, more preferably exhibits at least 70% sequence similarity to human EphA2, yet more preferably exhibits at least about 75% sequence similarity human EphA2. In other embodiments, the EphA2 polypeptide sequence preferably comprises an amino acid sequence that exhibits at least 85% sequence similarity to human EphA2, yet more preferably exhibits at least 90% sequence similarity to human EphA2, and most preferably exhibits at least about 95% sequence similarity to human EphA2.

[00128] Additional polypeptides suitable in the present methods are those encoded by the nucleic acids described in Section 5.2 below.

[00129] The determination of percent identity between two sequences can be accomplished using a mathematical algorithm. A preferred, non-limiting example of a mathematical algorithm utilized for the comparison of two sequences is the algorithm of Karlin and Altschul, 1990, *Proc Natl Acad Sci. USA* 87:2264-2268, modified as in Karlin and Altschul, 1993, *Proc Natl Acad Sci. USA* 90:5873-5877. Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul *et al.*,1990, *J. Mol. Biol.* 215:403-410. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to a nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to a protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.*, 1997, *Nucleic Acids Res.* 25:3389-3402. Alternatively, PSI-Blast can be used to perform an iterated search which detects distant relationships between molecules (*Id.*). When utilizing BLAST, Gapped BLAST, and PSI-Blast programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used.

[00130] Another preferred, non limiting example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller, 1988, *CABIOS* 4:11 17. Such an algorithm is incorporated into the ALIGN program (version 2.0) which is part of the GCG sequence alignment software package. When utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used. Additional algorithms for sequence analysis are known in the art and include ADVANCE and ADAM as described in Torellis and Robotti, 1994, *Comput. Appl. Biosci.* 10:3-5; and FASTA described in Pearson and Lipman,1988, 85:2444-8. Within FASTA, ktup is a control option that sets the

sensitivity and speed of the search. If ktup = 2, similar regions in the two sequences being compared are found by looking at pairs of aligned residues; if ktup = 1, single aligned amino acids are examined. ktup can be set to 2 or 1 for protein sequences, or from 1 to 6 for DNA sequences. The default if ktup is not specified is 2 for proteins and 6 for DNA. For a further description of FASTA parameters, *see* http://bioweb.pasteur.fr/docs/man/man/fasta.1.html#sect2.

[00131] The percent identity between two sequences can be determined using techniques similar to those described above, with or without allowing gaps. In calculating percent identity, only exact matches are counted. However, conservative substitutions should be considered in evaluating sequences that have a low percent identity with the EphA2 sequences disclosed herein.

[00132] In a specific embodiment, EphA2 antigenic polypeptides comprising at least 10, 20, 30, 40, 50, 75, 100, or 200 amino acids of an EphA2 polypeptide of SEQ ID NO:2 are used in the present invention. In a preferred embodiment, such a polypeptide comprises all or a portion of the extracellular domain of an EphA2 polypeptide of SEQ ID NO:2.

5.2 Methods of Identifying EphA2 Antigenic Peptides

5.2.1 Assays for EphA2 Antigenic Peptides

[00133] The present invention provides *Listeria*-based EphA2 vaccines comprising *Listeria* bacteria engineered to express an EphA2 antigenic peptide. Any assay known in the art for determining whether a peptide is a T cell epitope or a B cell epitope may be employed in testing EphA2 peptides for suitability in the present methods and compositions.

[00134] For example, ELISPOT assays and methods for intracellular cytokine staining can be used for enumeration and characterization of antigen-specific $CD4^+$ and $CD8^+$ T cells. Lalvani *et al.* (1997) *J. Exp. Med.* 186:859-865; Waldrop *et al.* (1997) *J. Clin Invest.* 99:1739-1750.

[00135] EphA2 antigenic peptides can be determined by screening synthetic peptides corresponding to portions of EphA2. Candidate antigenic peptides can be identified on the basis of their sequence or predicted structure. A number of algorithms are available for this purpose.

[00136] Exemplary protocols for such assays are presented below.

5.2.2 Peptides That Display Immunogenicity of EphA2

[00137] The ability of EphA2 peptides to elicit EphA2-specific antibody responses in mammals can be examined, for example, by immunizing animals (*e.g.*, mice, guinea pigs or rabbits) with individual EphA2 peptides emulsified in Freund's adjuvant.

[00138] After three injections (5 to 100 μg peptide per injection), IgG antibody responses are tested by peptide-specific ELISAs and immunoblotting against EphA2.

[00139] EphA2 peptides which produce antisera that react specifically with the EphA2 peptides and also recognized full length EphA2 protein in immunoblots are said to display the antigenicity of EphA2.

5.2.3 $CD4^+$ T-Cell Proliferation Assay

[00140] For example, such assays include *in vitro* cell culture assays in which peripheral blood mononuclear cells ("PBMCs") are obtained from fresh blood of a patient with a disease involving overexpression of EphA2, and purified by centrifugation using FICOLL-PLAQUE PLUS (Pharmacia, Upsalla, Sweden) essentially as described by Kruse and Sebald, 1992, *EMBO J.* 11:3237-3244. The peripheral blood mononuclear cells are incubated for 7-10 days with candidate EphA2 antigenic peptides. Antigen presenting cells may optionally be added to the culture 24 to 48 hours prior to the assay, in order to process and present the antigen. The cells are then harvested by centrifugation, and washed in RPMI 1640 media (GibcoBRL, Gaithersburg, MD). 5×10^4 activated T cells/well are in RPMI 1640 media containing 10% fetal bovine serum, 10 mM HEPES, ph 7.5, 2 mM L-glutamine, 100 units/ml penicillin G, and 100 μg/ml streptomycin sulphate in 96 well plates for 72 hrs at 37°C, pulsed with 1 μCi ^{3}H-thymidine (DuPont NEN, Boston, MA)/well for 6 hrs, harvested, and radioactivity measured in a TOPCOUNT scintillation counter (Packard Instrument Col., Meriden, CT).

5.2.4 Intracellular Cytokine Staining (ICS)

[00141] Measurement of antigen-specific, intracellular cytokine responses of T cells can be performed essentially as described by Waldrop *et al.*, 1997, *J. Clin. Invest.* 99:1739-1750; Openshaw *et al.*, 1995, *J. Exp. Med.* 182:1357-1367; or Estcourt *et al.*, 1997, *Clin. Immunol. Immunopathol.* 83:60-67. Purified PBMCs from patients with a disease involving EphA2-overexpressing cells are placed in 12x75 millimeter polystyrene tissue culture tubes (Becton Dickinson, Lincoln Park, N.J.) at a concentration of 1×10^6 cells per tube. A solution comprising 0.5 milliliters of HL-1 serum free medium, 100 units per milliliter of penicillin, 100 units per milliliter streptomycin, 2 millimolar L glutamine (Gibco BRL), varying amounts of individual EphA2 antigenic candidate peptides, and 1 unit of anti-CD28 mAb (Becton-Dickinson, Lincoln Park, N.J.) is added to each tube. Anti-CD3 mAb is added to a duplicate set of normal PBMC cultures as positive control. Culture tubes are

incubated for 1 hour. Brefeldin A is added to individual tubes at a concentration of 1 microgram per milliliter, and the tubes are incubated for an additional 17 hours.

[00142] PBMCs stimulated as described above are harvested by washing the cells twice with a solution comprising Dulbecco's phosphate-buffered saline (dPBS) and 10 units of Brefeldin A. These washed cells are fixed by incubation for 10 minutes in a solution comprising 0.5 milliliters of 4% paraformaldehyde and dPBS. The cells are washed with a solution comprising dPBS and 2% fetal calf serum (FCS). The cells are then either used immediately for intracellular cytokine and surface marker staining or are frozen for no more than three days in freezing medium, as described (Waldrop *et al.*, 1997, *J. Clin. Invest.* 99:1739-1750).

[00143] The cell preparations were rapidly thawed in a 37°C water bath and washed once with dPBS. Cells, either fresh or frozen, are resuspended in 0.5 milliliters of permeabilizing solution (Becton Dickinson Immunocytometry systems, San Jose, Calif.) and incubated for 10 minutes at room temperature with protection from light. Permeabilized cells are washed twice with dPBS and incubated with directly conjugated mAbs for 20 minutes at room temperature with protection from light. Optimal concentrations of antibodies are predetermined according to standard methods. After staining, the cells were washed, refixed by incubation in a solution comprising dPBS 1% paraformaldehyde, and stored away from light at 4°C for flow cytometry analysis.

5.2.5 ELISPOT Assays

[00144] The ELISPOT assay measures Th1-cytokine specific induction in murine splenocytes following *Listeria* vaccination. ELISPOT assays are performed to determine the frequency of T lymphocytes in response to endogenous antigenic peptide stimulation, and are as described in Geginat, et al., 2001, *J. Immunol.* 166:1877-1884. Balb/c mice (3 per group) are vaccinated with *L. monocytogenes* expressing candidate EphA2 antigenic peptides or HBSS as control. Whole mouse spleens are harvested and pooled five days after vaccination. Single cell suspensions of murine splenocytes are plated in the presence of various antigens overnight in a 37°C incubator.

[00145] Assays are performed in nitrocellulose-backed 96-well microtiter plates coated with rat anti-mouse IFN-γ mAb. For the testing of the candidate EphA2 antigenic peptide, a 1×10^{-5} M peptide solution is prepared. In round-bottom 96-well microtiter plates per well 6×10^5 unseparated splenocytes in 135 µl culture medium (α modification of Eagle's medium (Life Technologies, Eggenstein, Germany) supplemented with 10% FCS, 100 U/ml penicillin, 100 µg/ml streptomycin, 1×10^{-5} M 2-ME, and 2 mM glutamine) are mixed with 15 µl of the 1×10^{-5} M peptide solution to yield a final peptide concentration of

1×10^{-6} M. After 6 h of incubation at 37°C, cells are resuspended by vigorous pipetting, and 100 µl or 10 µl of cell suspension (4×10^5/well or 4×10^4/well, respectively) is transferred to Ab-coated ELISPOT plates and incubated overnight at 37°C. In the ELISPOT plates, the final volume was adjusted to 150 µl to ensure homogenous distribution of cells.

[00146] Purified $CD4^+$ or $CD8^+$ T cells are tested in a modified assay as follows: 15 µl prediluted peptide (1×10^{-5} M) is directly added to Ab-coated ELISPOT plates and mixed with 4×10^5 splenocytes from nonimmune animals as APC to yield a final volume of 100 µl. After 4 h of preincubation of APC at 37°C, 1×10^5 $CD4^+$ or $CD8^+$ cells purified from *L. monocytogenes*-immune mice are added per well in a volume of 50 µl and plates are incubated overnight at 37°C. The ELISPOT-based *ex vivo* MHC restriction analysis is performed after loading of cell lines expressing specific MHC class I molecules with 1×10^{-6} M peptide for 2 h at 37°C. Subsequently, unbound peptides are washed off (four times) to prevent binding of peptides to responder splenocytes. Per well of the ELISPOT plate, 1×10^5 peptide-loaded APC are mixed with 4×10^5 or 4×10^4 responder splenocytes in a final volume of 150 µl. After overnight incubation at 37°C, ELISPOT plates are developed with biotin-labeled rat anti-mouse IFN-γ mAb, HRP streptavidin conjugate, and aminoethylcarbazole dye of spots per splenocytes seeded. The specificity and sensitivity of the ELISPOT assay is controlled with IFN-γ secreting CD8 T cell lines specific for a control antigen.

5.3 Fusion Proteins

[00147] In certain embodiments of the present invention, an EphA2 vaccine comprises, expresses or is an antigen-presenting cell that is sensitized with an EphA2 antigenic peptide that is a fusion protein. Thus, the present invention encompasses compositions and methods in which the EphA2 antigenic peptides are fusion proteins comprising all or a fragment or derivative of EphA2 operatively associated to a heterologous component, *e.g.*, a heterologous peptide. Heterologous components can include, but are not limited to sequences which facilitate isolation and purification of the fusion protein. Heterologous components can also include sequences which confer stability to EphA2 antigenic peptides. Such fusion partners are well known to those of skill in the art.

[00148] The present invention encompasses the use of fusion proteins comprising an EphA2 polypeptide (*e.g.*, a polypeptide of SEQ ID NO:2) and a heterologous polypeptide (*i.e.*, an unrelated polypeptide or fragment thereof, preferably at least 10, at least 20, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90 or at least 100 amino

acids of the polypeptide). The fusion can be direct, but may occur through linker sequences. The heterologous polypeptide may be fused to the N-terminus or C-terminus of the EphA2 antigenic polypeptide. Alternatively, the heterologous polypeptide may be flanked by EphA2 polypeptide sequences.

[00149] A fusion protein can comprise an EphA2 antigenic polypeptide fused to a heterologous signal sequence at its N-terminus. Various signal sequences are commercially available. Eukaryotic heterologous signal sequences include, but are not limited to, the secretory sequences of melittin and human placental alkaline phosphatase (Stratagene, La Jolla, CA). Prokaryotic heterologous signal sequences useful in the methods of the invention include, but are not limited to, the phoA secretory signal (Sambrook *et al.*, eds., 1989, Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY) and the protein A secretory signal (Pharmacia Biotech, Piscataway, NJ).

[00150] The EphA2 antigenic polypeptide can be fused to tag sequences, *e.g.*, a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., Chatsworth, CA), among others, many of which are commercially available for use in the methods of the invention. As described in Gentz *et al.*, 1989, *Proc. Natl. Acad. Sci. USA*, 86:821-824, for instance, hexa-histidine provides for convenient purification of the fusion protein. Other examples of peptide tags are the hemagglutinin "HA" tag, which corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson *et al.*, 1984, *Cell*, 37:767) and the "flag" tag (Knappik *et al.*, 1994, *Biotechniques*, 17(4):754-761). These tags are especially useful for purification of recombinantly produced EphA2 antigenic polypeptides.

[00151] Any fusion protein may be readily purified by utilizing an antibody specific or selective for the fusion protein being expressed. For example, a system described by Janknecht *et al.* allows for the ready purification of non-denatured fusion proteins expressed in human cell lines (Janknecht *et al.*, 1991, *Proc. Natl. Acad. Sci. USA* 88:8972). In this system, the gene of interest is subcloned into a vaccinia recombination plasmid such that the open reading frame of the gene is translationally fused to an amino-terminal tag consisting of six histidine residues. Extracts from cells infected with recombinant vaccinia virus are loaded onto Ni^{2+} nitriloacetic acid-agarose columns and histidine-tagged proteins are selectively eluted with imidazole-containing buffers.

[00152] An affinity label can also be fused at its amino terminal to the carboxyl terminal of the EphA2 antigenic polypeptide for use in the methods of the invention. The precise site at which the fusion is made in the carboxyl terminal is not critical. The optimal site can be determined by routine experimentation. An affinity label can also be fused at its

carboxyl terminal to the amino terminal of the EphA2 antigenic polypeptide for use in the methods and compositions of the invention.

[00153] A variety of affinity labels known in the art may be used, such as, but not limited to, the immunoglobulin constant regions (*see also* Petty, 1996, Metal-chelate affinity chromatography, in Current Protocols in Molecular Biology, Vol. 2, Ed. Ausubel *et al.*, Greene Publish. Assoc. & Wiley Interscience), glutathione S-transferase (GST; Smith, 1993, *Methods Mol. Cell Bio.* 4:220-229), the *E. coli* maltose binding protein (Guan *et al.*, 1987, *Gene* 67:21-30), and various cellulose binding domains (U.S. Patent Nos. 5,496,934; 5,202,247; 5,137,819; Tomme *et al.*, 1994, *Protein Eng.* 7:117-123), *etc.* Other affinity labels are recognized by specific binding partners and thus facilitate isolation by affinity binding to the binding partner which can be immobilized onto a solid support. Some affinity labels may afford the EphA2 antigenic polypeptide novel structural properties, such as the ability to form multimers. These affinity labels are usually derived from proteins that normally exist as homopolymers. Affinity labels such as the extracellular domains of CD8 (Shiue *et al.*, 1988, *J. Exp. Med.* 168:1993-2005), or CD28 (Lee *et al.*, 1990, *J. Immunol.* 145:344-352), or fragments of the immunoglobulin molecule containing sites for interchain disulfide bonds, could lead to the formation of multimers.

[00154] As will be appreciated by those skilled in the art, many methods can be used to obtain the coding region of the above-mentioned affinity labels, including but not limited to, DNA cloning, DNA amplification, and synthetic methods. Some of the affinity labels and reagents for their detection and isolation are available commercially.

[00155] In certain embodiments, the affinity label is a non-variable portion of the immunoglobulin molecule. Typically, such portions comprise at least a functionally operative CH2 and CH3 domain of the constant region of an immunoglobulin heavy chain. Fusions are also made using the carboxyl terminus of the Fc portion of a constant domain, or a region immediately amino-terminal to the CH1 of the heavy or light chain. Suitable immunoglobulin-based affinity label may be obtained from IgG-1, -2, -3, or -4 subtypes, IgA, IgE, IgD, or IgM, but preferably IgG1. Many DNA encoding immunoglobulin light or heavy chain constant regions are known or readily available from cDNA libraries. See, for example, Adams *et al.*, *Biochemistry*, 1980, 19:2711-2719; Gough *et al.*, 1980, *Biochemistry*, 19:2702-2710; Dolby *et al.*, 1980, *Proc. Natl. Acad. Sci. U.S.A.*, 77:6027-6031; Rice *et al.*, 1982, *Proc. Natl. Acad. Sci. U.S.A.*, 79:7862-7865; Falkner *et al.*, 1982, *Nature*, 298:286-288; and Morrison *et al.*, 1984, *Ann. Rev. Immunol*, 2:239-256. Because many immunological reagents and labeling systems are available for the detection of immunoglobulins, the EphA2 antigenic polypeptide-Ig fusion protein can readily be

detected and quantified by a variety of immunological techniques known in the art, such as the use of enzyme-linked immunosorbent assay (ELISA), immunoprecipitation, fluorescence activated cell sorting (FACS), *etc.* Similarly, if the affinity label is an epitope with readily available antibodies, such reagents can be used with the techniques mentioned above to detect, quantitate, and isolate the EphA2 antigenic polypeptide containing the affinity label. In many instances, there is no need to develop specific or selective antibodies to the EphA2 antigenic polypeptide for the purposes of purification.

[00156] A fusion protein can comprise an EphA2 antigenic polypeptide fused to the Fc domain of an immunoglobulin molecule or a fragment thereof for use in the methods and compositions of the invention. A fusion protein can also comprise an EphA2 antigenic polypeptide fused to the CH2 and/or CH3 region of the Fc domain of an immunoglobulin molecule. Furthermore, a fusion protein can comprise an EphA2 antigenic polypeptide fused to the CH2, CH3, and hinge regions of the Fc domain of an immunoglobulin molecule (see Bowen *et al.*, 1996, *J. Immunol.* 156:442-49). This hinge region contains three cysteine residues which are normally involved in disulfide bonding with other cysteines in the Ig molecule. Since none of the cysteines are required for the peptide to function as a tag, one or more of these cysteine residues may optionally be substituted by another amino acid residue, such as for example, serine.

[00157] Various leader sequences known in the art can be used for the efficient secretion of the EphA2 antigenic polypeptide from bacterial and mammalian cells (von Heijne, 1985, J. *Mol. Biol.* 184:99-105). Leader peptides are selected based on the intended host cell, and may include bacterial, yeast, viral, animal, and mammalian sequences. For example, the herpes virus glycoprotein D leader peptide is suitable for use in a variety of mammalian cells. A preferred leader peptide for use in mammalian cells can be obtained from the V-J2-C region of the mouse immunoglobulin kappa chain (Bernard *et al.*, 1981, *Proc. Natl. Acad. Sci.* 78:5812-5816). Preferred leader sequences for targeting EphA2 antigenic polypeptide expression in bacterial cells include, but are not limited to, the leader sequences of the *E.coli* proteins OmpA (Hobom *et al.*, 1995, *Dev. Biol. Stand.* 84:255-262), Pho A (Oka *et al.*, 1985, *Proc. Natl. Acad. Sci* 82:7212-16), OmpT (Johnson *et al.*, 1996, *Protein Expression* 7:104-113), LamB and OmpF (Hoffman & Wright, 1985, *Proc. Natl. Acad. Sci. USA* 82:5107-5111), β-lactamase (Kadonaga *et al.*, 1984, *J. Biol. Chem.* 259:2149-54), enterotoxins (Morioka-Fujimoto *et al.*, 1991, *J. Biol. Chem.* 266:1728-32), and the *Staphylococcus aureus* protein A (Abrahmsen *et al.*, 1986, *Nucleic Acids Res.* 14:7487-7500), and the *B. subtilis* endoglucanase (Lo *et al.*, *Appl. Environ. Microbiol.*

54:2287-2292), as well as artificial and synthetic signal sequences (MacIntyre *et al.*, 1990, *Mol. Gen. Genet.* 221:466-74; Kaiser *et al.*, 1987, *Science*, 235:312-317).

[00158] Fusion proteins can be produced by standard recombinant DNA techniques or by protein synthetic techniques, *e.g.*, by use of a peptide synthesizer. For example, a nucleic acid molecule encoding a fusion protein can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers which give rise to complementary overhangs between two consecutive gene fragments which can subsequently be annealed and reamplified to generate a chimeric gene sequence (*see, e.g., Current Protocols in Molecular Biology*, Ausubel *et al.*, eds., John Wiley & Sons, 1992).

[00159] The nucleotide sequence coding for a fusion protein can be inserted into an appropriate expression vector, *i.e.*, a vector which contains the necessary elements for the transcription and translation of the inserted protein-coding sequence. The expression of a fusion protein may be regulated by a constitutive, inducible or tissue-specific or -selective promoter. It will be understood by the skilled artisan that fusion proteins, which can facilitate solubility and/or expression, and can increase the *in vivo* half-life of the EphA2 antigenic polypeptide and thus are useful in the methods of the invention. The EphA2 antigenic polypeptides or peptide fragments thereof, or fusion proteins can be used in any assay that detects or measures EphA2 antigenic polypeptides or in the calibration and standardization of such assay.

[00160] The methods of invention encompass the use of EphA2 antigenic polypeptides or peptide fragments thereof, which may be produced by recombinant DNA technology using techniques well known in the art. Thus, methods for preparing the EphA2 antigenic polypeptides of the invention by expressing nucleic acid containing EphA2 antigenic gene sequences are described herein. Methods which are well known to those skilled in the art can be used to construct expression vectors containing, *e.g.*, EphA2 antigenic polypeptide coding sequences (including but not limited to nucleic acids encoding all or an antigenic portion of a polypeptide of SEQ ID NO:2) and appropriate transcriptional and translational control signals. These methods include, for example, *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. See, for example, the techniques described in Sambrook *et al.*, 1989, *supra*, and Ausubel *et al.*, 1989, *supra*. Alternatively, RNA capable of encoding EphA2 antigenic polypeptide sequences may be chemically synthesized using, for example, synthesizers (*see, e.g.*, the techniques described in *Oligonucleotide Synthesis*, 1984, Gait, M.J. ed., IRL Press, Oxford).

[00161] In certain embodiments, the EphA2 antigenic polypeptide is functionally coupled to an internalization signal peptide, also referred to as a "protein transduction domain," that would allow its uptake into the cell nucleus. In certain specific embodiments, the internalization signal is that of Antennapedia (reviewed by Prochiantz, 1996, Curr. Opin. Neurobiol. 6:629 634, Hox A5 (Chatelin *et al.*, 1996, *Mech. Dev.* 55:111 117), HIV TAT protein (Vives *et al.*, 1997, *J. Biol. Chem.* 272:16010 16017) or VP22 (Phelan *et al.*, 1998, *Nat. Biotechnol.* 16:440 443).

5.4 Polynucleotides Encoding An EphA2 Antigenic Peptide

[00162] The present invention also encompasses compositions and methods that employ an EphA2 antigenic peptide expression vehicle.

[00163] In certain embodiments, the expression vehicles comprise or contain polynucleotides that hybridize under high stringency, intermediate or lower stringency hybridization conditions, *e.g.*, as defined *infra*, to polynucleotides that encode an EphA2 of the invention.

[00164] By way of example and not limitation, procedures using such conditions of low stringency for regions of hybridization of over 90 nucleotides are as follows (*see also* Shilo and Weinberg, 1981, *Proc. Natl. Acad. Sci. USA* 78:6789-6792). Filters containing DNA are pretreated for 6 hours at 40°C in a solution containing 35% formamide, 5X SSC, 50 mM Tris-HCl (pH 7.5), 5 mM EDTA, 0.1% PVP, 0.1% Ficoll, 1% BSA, and 500 μg/ml denatured salmon sperm DNA. Hybridizations are carried out in the same solution with the following modifications: 0.02% PVP, 0.02% Ficoll, 0.2% BSA, 100 μg/ml salmon sperm DNA, 10% (wt/vol) dextran sulfate, and 5-20 X 10^6 cpm ^{32}P-labeled probe is used. Filters are incubated in hybridization mixture for 18-20 h at 40°C, and then washed for 1.5 h at 55°C in a solution containing 2X SSC, 25 mM Tris-HCl (pH 7.4), 5 mM EDTA, and 0.1% SDS. The wash solution is replaced with fresh solution and incubated an additional 1.5 h at 60°C. Filters are blotted dry and exposed for autoradiography. If necessary, filters are washed for a third time at 65-68°C and re-exposed to film. Other conditions of low stringency which may be used are well known in the art (*e.g.*, as employed for cross-species hybridizations).

[00165] Also, by way of example and not limitation, procedures using such conditions of high stringency for regions of hybridization of over 90 nucleotides are as follows. Prehybridization of filters containing DNA is carried out for 8 h to overnight at 65°C in buffer composed of 6X SSC, 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.02% BSA, and 500 μg/ml denatured salmon sperm DNA. Filters are hybridized for 48 h at 65°C in prehybridization mixture containing 100 μg/ml denatured

salmon sperm DNA and 5-20 X 10^6 cpm of ^{32}P-labeled probe. Washing of filters is done at 37°C for 1 h in a solution containing 2X SSC, 0.01% PVP, 0.01% Ficoll, and 0.01% BSA. This is followed by a wash in 0.1X SSC at 50°C for 45 min before autoradiography.

[00166] Other conditions of high stringency which may be used depend on the nature of the nucleic acid (*e.g.*, length, GC content, *etc.*) and the purpose of the hybridization (detection, amplification, *etc.*) and are well known in the art. For example, stringent hybridization of a nucleic acid of approximately 15-40 bases to a complementary sequence in the polymerase chain reaction (PCR) is done under the following conditions: a salt concentration of 50 mM KCl, a buffer concentration of 10 mM Tris-HCl, a Mg^{2+} concentration of 1.5 mM, a pH of 7-7.5 and an annealing temperature of 55-60°C.

[00167] Selection of appropriate conditions for moderate stringencies is also well known in the art (*see, e.g.*, Sambrook *et al.*, 1989, Molecular Cloning, A Laboratory Manual, 2d Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York; *see also*, Ausubel *et al.*, eds., in the Current Protocols in Molecular Biology series of laboratory technique manuals, © 1987-1997, Current Protocols, © 1994-1997 John Wiley and Sons, Inc.).

[00168] The nucleic acids useful in the present methods may be made by any method known in the art. For example, if the nucleotide sequence of the EphA2 antigenic peptide is known, a nucleic acid encoding the peptide may be assembled from chemically synthesized oligonucleotides (*e.g.*, as described in Kutmeier *et al.*, 1994, *BioTechniques* 17:242), which, briefly, involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding the peptide, annealing and ligating of those oligonucleotides, and then amplification of the ligated oligonucleotides by PCR.

[00169] Alternatively, a polynucleotide encoding an EphA2 antigenic peptide may be generated from nucleic acid from a suitable source. If a clone containing a nucleic acid encoding a particular peptide is not available, but the sequence of the EphA2 antigenic peptide is known, a nucleic acid encoding the peptide may be chemically synthesized or obtained from a suitable source (*e.g.*, a cDNA library, or a cDNA library generated from, or nucleic acid, preferably poly A+ RNA, isolated from, any tissue or cells expressing EphA2) by PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence to identify, *e.g.*, a cDNA clone from a cDNA library that encodes the peptide. Amplified nucleic acids generated by PCR may then be cloned into replicable cloning vectors using any method well known in the art.

[00170] Further, a nucleic acid that is useful in the present methods may be manipulated using methods well known in the art for the manipulation of nucleotide sequences, *e.g.*, recombinant DNA techniques, site directed mutagenesis, PCR, *etc.* (see, for example, the techniques described in Sambrook *et al.*, 1990, Molecular Cloning, A Laboratory Manual, 2d Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY and Ausubel *et al.*, eds., 1998, Current Protocols in Molecular Biology, John Wiley & Sons, NY, which are both incorporated by reference herein in their entireties), to generate EphA2 antigenic peptides having a different amino acid sequence from the amino acid sequence depicted in SEQ ID NO:2, for example to create amino acid substitutions, deletions, and/or insertions.

5.5 Recombinant Expression Of An EphA2 antigenic peptide

[00171] Recombinant expression of an EphA2 antigenic peptide of the invention, or fragment or derivative thereof, requires construction of an expression vector containing a polynucleotide that encodes the EphA2 antigenic peptide. Once a polynucleotide encoding an EphA2 antigenic peptide of the invention has been obtained, the vector for the production of the EphA2 antigenic peptide may be produced by recombinant DNA technology using techniques well known in the art. Thus, methods for preparing a protein by expressing a polynucleotide containing an EphA2 antigenic peptide-encoding nucleotide sequence are described herein. Methods which are well known to those skilled in the art can be used to construct expression vectors containing peptide coding sequences and appropriate transcriptional and translational control signals. These methods include, for example, *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. The invention, thus, provides replicable vectors comprising a nucleotide sequence encoding an EphA2 antigenic peptide of the invention.

[00172] The expression vector is transferred to a host cell by conventional techniques and the transfected cells are then cultured by conventional techniques to produce an EphA2 antigenic peptide of the invention. Thus, the invention includes host cells containing a polynucleotide encoding an EphA2 antigenic peptide of the invention or fragments thereof, operably linked to a heterologous promoter.

[00173] A variety of host-expression vector systems may be utilized to express the EphA2 antigenic peptides of the invention (see, *e.g.*, U.S. Patent No. 5,807,715). Such host-expression systems represent vehicles by which the coding sequences of interest may be produced and subsequently purified, but also represent cells which may, when transformed or transfected with the appropriate nucleotide coding sequences, express an EphA2 antigenic peptide of the invention *in situ*. These include but are not limited to

microorganisms such as bacteria (*e.g.*, *E. coli* and *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing peptide coding sequences; yeast (*e.g.*, *Saccharomyces Pichia*) transformed with recombinant yeast expression vectors containing peptide coding sequences; insect cell systems infected with recombinant virus expression vectors (*e.g.*, baculovirus) containing peptide coding sequences; plant cell systems infected with recombinant virus expression vectors (*e.g.*, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (*e.g.*, Ti plasmid) containing peptide coding sequences; or mammalian cell systems (*e.g.*, COS, CHO, BHK, 293, NS0, and 3T3 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (*e.g.*, metallothionein promoter) or from mammalian viruses (*e.g.*, the adenovirus late promoter; the vaccinia virus 7.5K promoter). Preferably, bacterial cells such as *E. coli*, and more preferably, eukaryotic cells, especially for the expression of whole recombinant EphA2 antigenic peptide, are used for the expression of a recombinant EphA2 antigenic peptide. For example, mammalian cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus is an effective expression system for peptides (Foecking *et al.*, 1986, *Gene* 45:101; and Cockett *et al.*, 1990, *BioTechnology* 8:2). In a specific embodiment, the expression of nucleotide sequences encoding an EphA2 antigenic peptide of the invention is regulated by a constitutive promoter, inducible promoter or tissue specific promoter.

[00174] In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the EphA2 antigenic peptide being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of an EphA2 antigenic peptide vaccine, vectors which direct the expression of high levels of protein products that are readily purified may be desirable. Such vectors include, but are not limited to, the *E. coli* expression vector pUR278 (Ruther *et al.*, 1983, *EMBO* 12:1791), in which the peptide coding sequence may be ligated individually into the vector in frame with the lac Z coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, 1985, *Nucleic Acids Res.* 13:3101-3109; Van Heeke & Schuster, 1989, *J. Biol. Chem.* 24:5503-5509); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione 5-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption and binding to matrix glutathione-agarose beads followed by elution in the presence of free

glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

[00175] In an insect system, Autographa californica nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The peptide coding sequence may be cloned individually into non-essential regions (*e.g.*, the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (*e.g.*, the polyhedrin promoter).

[00176] In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the peptide coding sequence of interest may be ligated to an adenovirus transcription/translation control complex, *e.g.*, the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion in a non-essential region of the viral genome (*e.g.*, region El or E3) will result in a recombinant virus that is viable and capable of expressing the EphA2 antigenic peptide in infected hosts (*e.g.*, see Logan & Shenk, 1984, *PNAS* 8 1:355-359). Specific initiation signals may also be required for efficient translation of inserted peptide coding sequences. These signals include the ATG initiation codon and adjacent sequences. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see, *e.g.*, Bittner *et al.*, 1987, *Methods in Enzymol.* 153:516-544).

[00177] In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (*e.g.*, glycosylation) and processing (*e.g.*, cleavage) of protein products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include but are not limited to CHO, VERO, BHK, HeLa, COS, MDCK, 293, 3T3, W138, BT483, Hs578T, HTB2, BT2O, NS1 and

T47D, NS0 (a murine myeloma cell line that does not endogenously produce any peptide chains), CRL7O3O and HsS78Bst cells.

[00178] For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express the EphA2 antigenic peptide may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (*e.g.*, promoter, enhancer, sequences, transcription terminators, polyadenylation sites, *etc.*), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines which express the EphA2 antigenic peptide. Such engineered cell lines may be particularly useful in screening and evaluation of compositions that interact directly or indirectly with the EphA2 antigenic peptide.

[00179] A number of selection systems may be used, including but not limited to, the herpes simplex virus thymidine kinase (Wigler *et al.*, 1977, *Cell* 11:223), glutamine synthetase, hypoxanthine guanine phosphoribosyltransferase (Szybalski & Szybalski, 1992, *Proc. Natl. Acad. Sci. USA* 48:202), and adenine phosphoribosyltransferase (Lowy *et al.*, 1980, *Cell* 22:8-17) genes can be employed in tk-, gs-, hgprt- or aprt- cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: *dhfr*, which confers resistance to methotrexate (Wigler et al., 1980, *PNAS* 77:357; O'Hare et al., 1981, *PNAS* 78:1527); *gpt*, which confers resistance to mycophenolic acid (Mulligan & Berg, 1981, *PNAS* 78:2072); neo, which confers resistance to the aminoglycoside G-418 (Wu and Wu, 1991, *Biotherapy* 3:87; Tolstoshev, 1993, *Ann. Rev. Pharmacol. Toxicol.* 32:573; Mulligan, 1993, *Science* 260:926; and Morgan and Anderson, 1993, *Ann. Rev. Biochem.* 62: 191; May, 1993, *TIB TECH* 11:155-); and *hygro*, which confers resistance to hygromycin (Santerre et al., 1984, *Gene* 30:147). Methods commonly known in the art of recombinant DNA technology may be routinely applied to select the desired recombinant clone, and such methods are described, for example, in Ausubel et al. (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993); Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990); and in Chapters 12 and 13, Dracopoli et al. (eds), Current Protocols in Human Genetics, John Wiley & Sons, NY

(1994); Colberre-Garapin *et al.*, 1981, *J. Mol. Biol.* 150:1, which are incorporated by reference herein in their entireties.

[00180] The expression levels of an EphA2 antigenic peptide can be increased by vector amplification (for a review, see Bebbington and Hentschel, The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells in DNA cloning, Vol.3. (Academic Press, New York, 1987)). When a marker in the vector system expressing peptide is amplifiable, increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the peptide gene, production of the peptide will also increase (Crouse et al., 1983, *Mol. Cell. Biol.* 3:257).

[00181] Once an EphA2 antigenic peptide of the invention has been produced by recombinant expression, it may be purified by any method known in the art for purification of an EphA2 antigenic peptide, for example, by chromatography (*e.g.*, ion exchange, affinity, particularly by affinity for the specific antigen after Protein A, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. Further, the EphA2 peptides of the present invention or fragments thereof may be fused to heterologous polypeptide sequences described herein or otherwise known in the art to facilitate purification.

5.6 Gene Therapy

[00182] As discussed above, the present invention encompasses compositions and methods employ an EphA2 antigenic peptide expression vehicles. In certain embodiments, the expression vehicle is any gene therapy vector available in the art can be used. Exemplary gene therapy vectors that may be used as EphA2 antigenic peptide expression vehicles are described below.

[00183] For general reviews of the methods of gene therapy, *see*, Goldspiel *et al.*, 1993, Clinical Pharmacy 12:488-505; Wu and Wu, 1991, Biotherapy 3:87-95; Tolstoshev, 1993, Ann. Rev. Pharmacol. Toxicol. 32:573-596; Mulligan, 1993, Science 260:926-932; Morgan and Anderson, 1993, Ann. Rev. Biochem. 62:191-217; May, 1993, TIBTECH 1, l(5):155-215. Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel *et al.* (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993); and Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990).

[00184] In a preferred aspect, the expression vehicle comprises nucleic acid sequences encoding an EphA2 antigenic peptide, said nucleic acid sequences being part of expression vectors that express the EphA2 antigenic peptide in a suitable host. In

particular, such nucleic acid sequences have promoters operably linked to the EphA2 antigenic peptide, said promoter being inducible or constitutive, and, optionally, tissue-specific. In another particular embodiment, nucleic acid molecules are used in which the EphA2 antigenic peptide coding sequences and any other desired sequences are flanked by regions that promote homologous recombination at a desired site in the genome, thus providing for intrachromosomal expression of the EphA2 antigenic peptide (Koller and Smithies, 1989, Proc. Natl. Acad. Sci. USA 86:8932-8935; Zijlstra *et al.*, 1989, Nature 342:435-438.

[00185] Delivery of the nucleic acids into a subject may be either direct, in which case the patient is directly exposed to the nucleic acid or nucleic acid-carrying vectors, or indirect, in which case, cells are first transformed with the nucleic acids *in vitro*, then transplanted into the patient. These two approaches are known, respectively, as *in vivo* or *ex vivo* gene therapy.

[00186] In a specific embodiment, the nucleic acid sequences are directly administered *in vivo*, where it is expressed to produce the encoded EphA2 antigenic peptide. This can be accomplished by any of numerous methods known in the art, for example by constructing them as part of an appropriate nucleic acid expression vector and administering the vector so that the nucleic acid sequences become intracellular. Gene therapy vectors can be administered by infection using defective or attenuated retrovirals or other viral vectors (*see, e.g.*, U.S. Patent No. 4,980,286); direct injection of naked DNA; use of microparticle bombardment (*e.g.*, a gene gun; Biolistic, Dupont); coating with lipids or cell-surface receptors or transfecting agents; encapsulation in liposomes, microparticles, or microcapsules; administration in linkage to a peptide which is known to enter the nucleus; administration in linkage to a ligand subject to receptor-mediated endocytosis (*see, e.g.*, Wu and Wu, 1987, J. Biol. Chem. 262:4429-4432) (which can be used to target cell types specifically expressing the receptors); *etc.* In another embodiment, nucleic acid-ligand complexes can be formed in which the ligand comprises a fusogenic viral peptide to disrupt endosomes, allowing the nucleic acid to avoid lysosomal degradation. In yet another embodiment, the nucleic acid can be targeted *in vivo* for cell specific uptake and expression, by targeting a specific receptor (*see, e.g.*, PCT Publications WO 92/06 180; WO 92/22635; W092/20316; W093/14188, and WO 93/20221). Alternatively, the nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression by homologous recombination (Koller and Smithies, 1989, Proc. Natl. Acad. Sci. USA 86:8932-8935; Zijlstra *et al.*, 1989, Nature 342:435-438).

[00187] In a specific embodiment, viral vectors that contain nucleic acid sequences encoding an EphA2 antigenic peptide. For example, a retroviral vector can be used (see Miller *et al.*, 1993, Meth. Enzymol. 217:581-599). These retroviral vectors contain the components necessary for the correct packaging of the viral genome and integration into the host cell DNA. The nucleic acid sequences encoding the EphA2 antigenic peptide to be used in gene therapy are cloned into one or more vectors, thereby facilitating delivery of the gene into a patient. More detail about retroviral vectors can be found in Boesen *et al.*, 1994, Biotherapy 6:291-302, which describes the use of a retroviral vector to deliver the mdr 1 gene to hematopoietic stem cells in order to make the stem cells more resistant to chemotherapy. Other references illustrating the use of retroviral vectors in gene therapy are: Clowes *et al.*, 1994, J. Clin. Invest. 93:644-651; Klein *et al.*, 1994, Blood 83:1467-1473; Salmons and Gunzberg, 1993, Human Gene Therapy 4:129-141; and Grossman and Wilson, 1993, Curr. Opin. in Genetics and Devel. 3:110-114.

[00188] One approach to gene therapy encompassed by the present methods and compositions involves transferring a gene, *e.g.*, a nucleic acid encoding an EphA2 antigenic peptide, to cells in tissue culture by such methods as electroporation, lipofection, calcium phosphate mediated transfection, or viral infection. Usually, the method of transfer includes the transfer of a selectable marker to the cells. The cells are then placed under selection to isolate those cells that have taken up and are expressing the transferred gene. Those cells are then delivered to a subject.

[00189] In this embodiment, the nucleic acid is introduced into a cell prior to administration *in vivo* of the resulting recombinant cell. Such introduction can be carried out by any method known in the art, including but not limited to transfection, electroporation, microinjection, infection with a viral or bacteriophage vector containing the nucleic acid sequences, cell fusion, chromosome-mediated gene transfer, microcell mediated gene transfer, spheroplast fusion, *etc.* Numerous techniques are known in the art for the introduction of foreign genes into cells (*see, e.g.*, Loeffler and Behr, 1993, Meth. Enzymol. 217:599-618; Cohen *et al.*, 1993, Meth. Enzymol. 217:618-644; Cline, 1985, Pharmac. Ther. 29:69-92) and may be used in accordance with the present invention, provided that the necessary developmental and physiological functions of the recipient cells are not disrupted. The technique should provide for the stable transfer of the nucleic acid to the cell, so that the nucleic acid is expressible by the cell and preferably heritable and expressible by its cell progeny.

[00190] The resulting recombinant cells can be delivered to a patient by various methods known in the art. Recombinant blood cells (*e.g.*, hematopoietic stem or progenitor

cells) are preferably administered intravenously. The amount of cells envisioned for use depends on the desired effect, patient state, *etc.*, and can be determined by one skilled in the art.

[00191] Cells into which a nucleic acid can be introduced for purposes of gene therapy encompass any desired, available cell type, and include but are not limited to fibroblasts; blood cells such as T lymphocytes, B lymphocytes, monocytes, macrophages, neutrophils, eosinophils, megakaryocytes, granulocytes; various stem or progenitor cells, in particular hematopoietic stem or progenitor cells, *e.g.*, as obtained from bone marrow, umbilical cord blood, peripheral blood, fetal liver, *etc.*

[00192] In a preferred embodiment, the cell used for gene therapy is autologous to the patient.

[00193] In an embodiment in which recombinant cells are used in gene therapy, nucleic acid sequences encoding EphA2 antigenic peptide are introduced into the cells such that they are expressible by the cells or their progeny, and the recombinant cells are then administered *in vivo* for therapeutic effect. In a specific embodiment, stem or progenitor cells are used. Any stem and/or progenitor cells which can be isolated and maintained *in vitro* can potentially be used in accordance with this embodiment of the present invention (see *e.g.* PCT Publication WO 94/08598; Stemple and Anderson, 1992, Cell 71:973-985; Rheinwald, 1980, Meth. Cell Bio. 21A:229; and Pittelkow and Scott, 1986, Mayo Clinic Proc. 61:771).

[00194] In a specific embodiment, the nucleic acid to be introduced for purposes of gene therapy comprises an inducible promoter operably linked to the coding region, such that expression of the nucleic acid is controllable by controlling the presence or absence of the appropriate inducer of transcription.

5.7 Bacterial Expression Vehicles

[00195] In certain embodiments, the present invention provides EphA2 antigenic peptide expression vehicles in the form of a microorganism, and, in specific embodiments, the microorganism is a bacterium.

[00196] Microorganisms useful for the methods of the present invention include but are not limited to *Borrelia burgdorferi*, *Brucella melitensis*, *Escherichia coli*, *enteroinvasive Escherichia coli*, *Legionella pneumophila*, *Salmonella typhi*, *Salmonella typhimurium*, *Shigella spp.*, *Streptococcus spp.*, *Treponema pallidum*, *Yersinia enterocohtica*, *Listeria monocytogenes*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium tuberculosis*, *BCG*, *Mycoplasma hominis*, *Rickettsiae quintana*, *Cryptococcus neoformans*, *Histoplasma capsulatum*, *Pneumocystis carnii*, *Eimeria*

acervulina, Neospora caninum, Plasmodium falciparum, Sarcocystis suihominis, Toxoplasma gondii, Leishmania amazonensis, Leishmania major, Leishmania mexacana, Leptomonas karyophilus, Phytomonas spp., Trypanasoma cruzi, Encephalitozoon cuniculi, Nosema helminthorum, Unikaryon legeri.

[00197] Many of the microorganisms encompassed by the present invention are causative agents of diseases in humans and animals. For example, sepsis from gram negative bacteria is a serious problem because of the high mortality rate associated with the onset of septic shock (R.C. Bone, 1993, Clinical Microbiol. Revs. 6:57-68). Therefore, to allow the safe use of these microorganisms in both diagnostics and treatment of humans and animals, the microorganisms are attenuated in their virulence for causing disease. The end result is to reduce the risk of toxic shock or other side effects due to administration of the vector to the patient. Such attenuated microorganisms can be isolated by a number of techniques. Such methods include use of antibiotic-sensitive strains of microorganisms, mutagenesis of the microorganisms, selection for microorganism mutants that lack virulence factors, and construction of new strains of microorganisms with altered cell wall lipopolysaccharides.

[00198] In certain embodiments, the microorganisms can be attenuated by the deletion or disruption of DNA sequences which encode for virulence factors which insure survival of the microorganisms in the host cell, especially macrophages and neutrophils, by, for example, homologous recombination techniques and chemical or transposon mutagenesis. Many, but not all, of these studied virulence factors are associated with survival in macrophages such that these factors are specifically expressed within macrophages due to stress, for example, acidification, or are used to induced specific host cell responses, for example, macropinocytosis, Fields et al., 1986, Proc. Natl. Acad. Sci. USA 83:5189-5193. Bacterial virulence factors include, for example: cytolysin; defensin resistance loci; DNA K; fimbriae; GroEL; inv loci; lipoprotein; LPS; lysosomal fusion inhibition; macrophage survival loci; oxidative stress response loci; pho loci (*e.g.*, PhoP and PhoQ); pho activated genes (pag; e.g., pagB and pagC); phoP and phoQ regulated genes (prg); porins; serum resistance peptide; virulence plasmids (such as spvB, traT and ty2).

[00199] Yet another method for the attenuation of the microorganisms is to modify substituents of the microorganism which are responsible for the toxicity of that microorganism. For example, lipopolysaccharide (LPS) or endotoxin is primarily responsible for the pathological effects of bacterial sepsis. The component of LPS which results in this response is lipid A (LA). Elimination or mitigation of the toxic effects of LA results in an attenuated bacteria since 1) the risk of septic shock in the patient would be

reduced and 2) higher levels of the bacterial EphA2 antigenic peptide expression vehicle could be tolerated.

[00200] *Rhodobacter* (Rhodopseudomonas) *sphaeroides* and *Rhodobacter capsulatus* each possess a monophosphoryl lipid A (MLA) which does not elicit a septic shock response in experimental animals and, further, is an endotoxin antagonist. Loppnow et al., 1990, Infect. Immun. 58:3743-3750; Takayma et al., 1989, Infect. Immun. 57:1336-1338. Gram negative bacteria other than *Rhodobacter* can be genetically altered to produce MLA, thereby reducing its potential of inducing septic shock.

[00201] Yet another example for altering the LPS of bacteria involves the introduction of mutations in the LPS biosynthetic pathway. Several enzymatic steps in LPS biosynthesis and the genetic loci controlling them in a number of bacteria have been identified, and several mutant bacterial strains have been isolated with genetic and enzymatic lesions in the LPS pathway. In certain embodiments, the LPS pathway mutant is a firA mutant. firA is the gene that encodes the enzyme UDP-3-O(R-30 hydroxymyristoyl)-glycocyamine N-acyltransferase, which regulates the third step in endotoxin biosynthesis (Kelley et al., 1993, J. Biol. Chem. 268:19866-19874).

[00202] As a method of insuring the attenuated phenotype and to avoid reversion to the non-attenuated phenotype, the bacteria may be engineered such that it is attenuated in more than one manner, *e.g.*, a mutation in the pathway for lipid A production and one or more mutations to auxotrophy for one or more nutrients or metabolites, such as uracil biosynthesis, purine biosynthesis, and arginine biosynthesis.

[00203] In certain embodiments of the present invention, the bacterial EphA2 antigenic peptide expression vehicles are engineered to deliver suicide genes to the target EphA2-expressing cells. These suicide genes include pro-drug converting enzymes, such as Herpes simplex thymidine kinase (TK) and bacterial cytosine deaminase (CD). TK phosphorylates the non-toxic substrates acyclovir and ganciclovir, rendering them toxic via their incorporation into genomic DNA. CD converts the non-toxic 5-fluorocytosine (5-FC) into 5-fluorouracil (5-FU), which is toxic via its incorporation into RNA. Additional examples of pro-drug converting enzymes encompassed by the present invention include cytochrome p450 NADPH oxidoreductase which acts upon mitomycin C and porfiromycin (Murray et al., 1994, J. Pharmacol. Exp. Therapeut. 270:645-649). Other exemplary pro-drug converting enzymes that may be used in the methods and compositions of the present invention include: carboxypeptidase; beta-glucuronidase; penicillin-V-amidase; penicillin-G-amidase; beta-lactamase; beta.-glucosidase; nitroreductase; and carboxypeptidase A.

[00204] Where the EphA2 vaccine comprises a microorganism that expresses an EphA2 antigenic peptide and, optionally, a pro-drug converting enzyme, the expression constructs are preferably designed such that the microorganism-produced peptides and enzymes are secreted by the microorganism. A number of bacterial secretion signals are well known in the art and may be used in the compositions and methods of the present invention. Exemplary secretion signals that can be used with gram-positive microorganisms include SecA (Sadaie et al., Gene 98:101-105, 1991), SecY (Suh et al., Mol. Microbiol. 4:305-314, 1990), SecE (Jeong *et al.*, Mol. Microbiol. 10:133-142, 1993), FtsY an FfH (PCT/NL 96/00278), and PrsA (WO 94/19471). Exemplary secretion signals that may be used with gram-negative microorganisms include those of soluble cytoplasmic proteins such as SecB and heat shock proteins; that of the peripheral membrane-associated protein SecA; and those of the integral membrane proteins SecY, SecE, SecD and SecF.

[00205] The promoters driving the expression of the EphA2 antigenic peptides and, optionally, pro-drug converting enzymes, may be either constitutive, in which the peptides or enzymes are continually expressed, inducible, in which the peptides or enzymes are expressed only upon the presence of an inducer molecule(s), or cell-type specific control, in which the peptides or enzymes are expressed only in certain cell types. For example, a suitable inducible promoter can a promoter responsible for the baterial "SOS" response (Friedberg et al., In: DNA Repair and Mutagenesis, pp. 407-455, Am. Soc. Microbiol. Press, 1995). Such a promoter is inducible by numerous agents including chemotherapeutic alkylating agents such as mitomycin (Oda et al., 1985, Mutation Research 147:219-229; Nakamura et al., 1987, Mutation Res. 192:239-246; Shimda et al., 1994, Carcinogenesis 15:2523-2529) which is approved for use in humans. Promoter elements which belong to this group include umuC, sulA and others (Shinagawa et al., 1983, Gene 23:167-174; Schnarr et al., 1991, Biochemie 73:423-431). The sulA promoter includes the ATG of the sulA gene and the following 27 nucleotides as well as 70 nucleotides upstream of the ATG (Cole, 1983, Mol. Gen. Genet. 189:400-404). Therefore, it is useful both in expressing foreign genes and in creating gene fusions for sequences lacking initiating codons.

5.7.1 Exemplary Embodiment: *Listeria monocytogenes* As An Expression Vehicle

[00206] *Listeria monocytogenes* (*Listeria*) is a Gram-positive facultative intracellular bacterium that is being developed for use in antigen-specific vaccines due to its ability to prime a potent CD4+/CD8+ T-cell mediated response via both MHC class I and class II antigen presentation pathways, and as such it has been tested recently as a vaccine vector in a human clinical trial among normal healthy volunteers.

[00207] *Listeria* has been studied for many years as a model for stimulating both innate and adaptive T cell-dependent antibacterial immunity. The ability of *Listeria* to effectively stimulate cellular immunity is based on its intracellular lifecycle. Upon infecting the host, the bacterium is rapidly taken up by phagocytes including macrophages and dendritic cells into a phagolysosomal compartment. The majority of the bacteria are subsequently degraded. Peptides resulting from proteolytic degradation of pathogens within phagosomes of infected APCs are loaded directly onto MHC class II molecules, and these MHC II-peptide complexes activate CD4+ "helper" T cells that stimulate the production of antibodies, and the processed antigens are expressed on the surface of the antigen presenting cell via the class II endosomal pathway. Within the acidic compartment, certain bacterial genes are activated including the cholesterol-dependent cytolysin, LLO, which can degrade the phagolysosome, releasing the bacterium into the cytosolic compartment of the host cell, where the surviving *Listeria* propagate. Efficient presentation of heterologous antigens via the MHC class I pathway requires de novo endogenous protein expression by *Listeria*. Within antigen presenting cells (APC), proteins synthesized and secreted by *Listeria* are sampled and degraded by the proteosome. The resulting peptides are shuttled into the endoplasmic reticulum by TAP proteins and loaded onto MHC class I molecules. The MHC I-peptide complex is delivered to the cell surface, which in combination with sufficient co-stimulation (signal 2) activates and stimulates cytotoxic T lymphocytes (CTLs) having the cognate T cell receptor to expand and subsequently recognize the MHC I-peptide complex.

[00208] The EphA2 antigenic peptides are preferably expressed in *Listeria* using a heterologous gene expression cassette. A heterologous gene expression cassette is typically comprised of the following ordered elements: (1) prokaryotic promoter; (2) Shine-Dalgarno sequence; (3) secretion signal (signal peptide); and, (4) heterologous gene. Optionally, the heterologous gene expression cassette may also contain a transcription termination sequence, in constructs for stable integration within the bacterial chromosome. While not required, inclusion of a transcription termination sequence as the final ordered element in a heterologous gene expression cassette may prevent polar effects on the regulation of expression of adjacent genes, due to read-through transcription.

[00209] The expression vectors introduced into the *Listeria*-based EphA2 vaccine are preferably designed such that the *Listeria*-produced EphA2 peptides and, optionally, prodrug converting enzymes, are secreted by the *Listeria*. A number of bacterial secretion signals are well known in the art and may be used in the compositions and methods of the present invention. Exemplary secretion signals that can be used with gram-positive

microorganisms include SecA (Sadaie *et al.*, 1991, *Gene* 98:101-105), SecY (Suh *et al.*, 1990, *Mol. Microbiol.* 4:305-314), SecE (Jeong *et al.*, 1993, *Mol. Microbiol.* 10:133-142), FtsY and FfH (PCT/NL 96/00278), and PrsA (WO 94/19471).

[00210] The promoters driving the expression of the EphA2 antigenic peptides and, optionally, pro-drug converting enzymes, may be either constitutive, in which the peptides or enzymes are continually expressed, inducible, in which the peptides or enzymes are expressed only upon the presence of an inducer molecule(s), or cell-type specific control, in which the peptides or enzymes are expressed only in certain cell types. For example, a suitable inducible promoter can be a promoter responsible for the bacterial "SOS" response (Friedberg *et al.*, In: DNA Repair and Mutagenesis, pp. 407-455, Am. Soc. Microbiol. Press, 1995). Such a promoter is inducible by numerous agents including chemotherapeutic alkylating agents such as mitomycin (Oda *et al.*, 1985, *Mutation Research* 147:219-229; Nakamura *et al.*, 1987, *Mutation Res.* 192:239-246; Shimda *et al.*, 1994, *Carcinogenesis* 15:2523-2529) which is approved for use in humans. Promoter elements which belong to this group include umuC, sulA and others (Shinagawa *et al.*, 1983, *Gene* 23:167-174; Schnarr *et al.*, 1991, *Biochemie* 73:423-431). The sulA promoter includes the ATG of the sulA gene and the following 27 nucleotides as well as 70 nucleotides upstream of the ATG (Cole, 1983, *Mol. Gen. Genet.* 189:400-404). Therefore, it is useful both in expressing foreign genes and in creating gene fusions for sequences lacking initiating codons.

[00211] Preferred embodiments of components of the EphA2 antigenic peptide expression system, to be used in conjunction with nucleic acids encoding EphA2 antigenic peptides described in Section 5.2, are provided below.

5.7.1.1. Construct Backbone

[00212] One of ordinary skill in the art will recognize that a variety of plasmid construct backbones are available which are suitable for use in the assembly of a heterologous gene expression cassette. A particular plasmid construct backbone is selected based on whether expression of the heterologous gene from the bacterial chromosome or from an extra-chromosomal episome is desired.

[00213] Given as non-limiting examples, incorporation of the heterologous gene expression cassette into the bacterial chromosome of *Listeria monocytogenes* (*Listeria*) is accomplished with an integration vector that contains an expression cassette for a listeriophage integrase that catalyzes sequence-specific integration of the vector into the *Listeria* chromosome. For example, the integration vectors known as pPL1 and pPL2 program stable single-copy integration of a heterologous protein (e.g., EphA2-antigenic peptide) expression cassette within an innocuous region of the bacterial genome, and have

been described in the literature (Lauer et. al.2002 J. Bacteriol. 184:4177-4178). The integration vectors are stable as plasmids in E. coli and are introduced via conjugation into the desired *Listeria* background. Each vector lacks a *Listeria*-specific origin of replication and encodes a phage integrase, such that the vectors are stable only upon integration into a chromosomal phage attachment site. Starting with a desired plasmid construct, the process of generating a recombinant *Listeria* strain expressing a desired protein(s) takes approximately one week. The pPL1 and pPL2 integration vectors are based, respectively, on the U153 and PSA listeriophages. The pPL1 vector integrates within the open reading frame of the comK gene, while pPL2 integrates within the tRNAArg gene in such a manner that the native sequence of the gene is restored upon successful integration, thus keeping its native expressed function intact. The pPL1 and pPL2 integration vectors contain a multiple cloning site sequence in order to facilitate construction of plasmids containing the heterologous protein (e.g., EphA2-antigenic peptide) expression cassette. Alternatively, incorporation of the EphA2-antigenic peptide expression cassette into the *Listeria* chromosome can be accomplished through alleleic exchange methods, known to those skilled in the art. In particular, compositions in which it is desired to not incorporate a gene encoding an antibiotic resistance protein as part of the construct containing the heterologous gene expression cassette, methods of allelic exchange are desirable. For example, the pKSV7 vector (Camilli et. al. Mol. Microbiol. 1993 8,143-157), contains a temperature-sensitive *Listeria* Gram-positive replication origin which is exploited to select for recombinant clones at the non-permissive temperature that represent the pKSV7 plasmid recombined into the *Listeria* chromosome. The pKSV7 allelic exchange plasmid vector contains a multiple cloning site sequence in order to facilitate construction of plasmids containing the heterologous protein (e.g., EphA2-antigenic peptide) expression cassette, and also a chloramphenicol resistance gene. For insertion into the *Listeria* chromosome, the heterologous EphA2-antigenic peptide expression cassette construct is optimally flanked by approximately 1 kb of chromosomal DNA sequence that corresponds to the precise location of desired integration. The pKSV7-heterologous protein (e.g., EphA2-antigenic peptide) expression cassette plasmid is introduced optimally into a desired bacterial strain by electroporation, according to standard methods for electroporation of Gram positive bacteria. Briefly, bacteria electroporated with the pKSV7-heterologous protein (e.g., EphA2-antigenic peptide) expression cassette plasmid are selected by plating on BHI agar media containing chloramphenicol (10 μg/ml), and incubated at the permissive temperature of 30oC. Single cross-over integration into the bacterial chromosome is selected by passaging several individual colonies for multiple generations at the non-permissive

temperature of 41oC in media containing chloramphenicol. Finally, plasmid excision and curing (double cross-over) is achieved by passaging several individual colonies for multiple generations at the permissive temperature of 30°C in BHI media not containing chloramphenicol. Verification of integration of the heterologous protein (e.g., EphA2-antigenic peptide) expression cassette into the bacteria chromosome can be accomplished by PCR, utilizing a primer pair that amplifies a region defined from within the heterologous protein (e.g., EphA2-antigenic peptide) expression cassette to the bacterial chromosome targeting sequence not contained in the pKSV7 plasmid vector construct.

[00214] In other compositions, it may be desired to express the heterologous protein (e.g., EphA2-antigenic peptide) from a stable plasmid episome. Maintenance of the plasmid episome through passaging for multiple generations requires the co-expression of a protein that confers a selective advantage for the plasmid-containing bacterium. As non-limiting examples, the protein co-expressed from the plasmid in combination with the heterologous protein (e.g., EphA2-antigenic peptide) may be an antibiotic resistance protein, for example chloramphenicol, or may be a bacterial protein (that is expressed from the chromosome in wild-type bacteria), that can also confer a selective advantage. Non-limiting examples of bacterial proteins include enzyme required for purine or amino acid biosynthesis (selection under defined media lacking relevant amino acids or other necessary precursor macromolecules), or a transcription factor required for the expression of genes that confer a selective advantage in vitro or in vivo (Gunn et. al. 2001 J. Immuol. 167:6471-6479). As a non-limiting example, pAM401 is a suitable plasmid for episomal expression of a selected heterologous protein (e.g., EphA2-antigenic peptide) in diverse Gram-positive bacterial genera (Wirth et. al. 1986 J. Bacteriol 165:831-836).

5.7.1.2. Shine-Dalgarno Sequence

[00215] At the 3' end of the promoter is contained a poly-purine Shine-Dalgarno sequence, the element required for engagement of the 30S ribosomal subunit (via 16S rRNA) to the heterologous gene RNA transcript and initiation of translation. The Shine-Dalgarno sequence has typically the following consensus sequence: (SEQ ID NO:66): 5'-NAGGAGGU-N5-10-AUG (start codon)-3'. There are variations of the poly-purine Shine-Dalgarno sequence Notably, the *Listeria* hly gene that encodes listerolysin O (LLO) has the following Shine-Dalgarno sequence (SEQ ID NO:67): AAGGAGAGTGAAACCC**ATG** (Shine-Dalgarno sequence is underlined, and the translation start codon is bolded).

5.7.1.3. Codon Optimization

[00216] It is well known to those skilled in the art that for optimal translation efficiency of a selected heterologous protein, it is desirable to utilize codons favored by the bacterium. The preferred codon usage for bacterial expression can be found at the following link: http://www.kazusa.or.jp/codon/. In some embodiments, expression of heterologous proteins from *Listeria* monocytogenes is desired. The preferred *Listeria* monocytogenes codon usage can be found at the following link: http://www.kazusa.or.jp/codon/cgi-bin/showcodon.cgi?species=*Listeria*+monocytogenes+[gbbct]

[00217] The optimal codons utilized by *Listeria* monocytogenes for each amino acid are shown in the table below.

Listeria Codon Bias: Codons to be used for optimizing expression

Amino Acid	One Letter Code	Optimal *Listeria* Codon
Alanine	A	GCA
Arginine	R	CGU
Asparagine	N	AAU
Aspartate	D	GAU
Cysteine	C	UGU
Glutamine	Q	CAA
Glutamate	E	GAA
Glycine	G	GGU
Histidine	H	CAU
Isoleucine	I	AUU
Leucine	L	UUA
Lysine	K	AAA
Methionine	M	AUG
Phenylalanine	F	UUU
Proline	P	CCA
Serine	S	AGU
Threonine	T	ACA
Tryptophan	W	UGG
Tyrosine	Y	UAU
Valine	V	GUU

5.7.1.4. Signal Peptides

[00218] Bacteria utilize diverse pathways for protein secretion, including secA1 and Twin-Arg Translocation (Tat), which are located at the N-terminal end of the pre-protein. The majority of secreted proteins utilize the Sec pathway, in which the protein translocates through the bacterial membrane-embedded proteinaceous Sec pore in an unfolded

conformation. In contrast, the proteins utilizing the Tat pathway are secreted in a folded conformation. Nucleotide sequence encoding signal peptides corresponding to either of these protein secretion pathways can be fused genetically in-frame to a desired heterologous protein coding sequence. The signal peptides optimally contain a signal peptidase at their carboxyl terminus for release of the authentic desired protein into the extra-cellular environment (Sharkov and Cai. 2002 J. Biol. Chem. 277:5796-5803; Nielsen et. al. 1997 Protein Engineering 10:1-6; and, http://www.cbs.dtu.dk/services/SignalP/). The signal peptides can be derived not only from diverse secretion pathways, but also from diverse bacterial genera. Signal peptides have a common structural organization, having a charged N-terminus (N-domain), a hydrophobic core region (H-domain) and a more polar C-terminal region (C-domain), however, they do not show sequence conservation. The C-domain of the signal peptide carries a type I signal peptidase (SPase I) cleavage site, having the consensus sequence A-X-A, at positions –1 and –3 relative to the cleavage site. Proteins secreted via the sec pathway have signal peptides that average 28 residues. Signal peptides related to proteins secreted by the Tat pathway have a tripartite organization similar to Sec signal peptides, but are characterized by having an RR-motif (R-R-X-#-#, where # is a hydrophobic residue), located at the N-domain / H-domain boundary. Bacterial Tat signal peptides average 14 amino acids longer than sec signal peptides. The Bacillus subtilis secretome may contain as many as 69 putative proteins that utilize the Tat secretion pathway, 14 of which contain a SPase I cleavage site (Jongbloed et. al. 2002 J. Biol. Chem. 277:44068-44078; Thalsma et. al., 2000 Microbiol. Mol. Biol. Rev. 64:515-547). Shown in the table below are non-limiting examples of signal peptides that can be used in fusion compositions with a selected heterologous gene, resulting in secretion from the bacterium of the encoded protein.

Secretion Pathway	Signal Peptide Amino Acid Sequence (NH_2-CO_2)	Signal peptidase Site (cleavage site represented by ')	Gene	Genus/species	SEQ ID NO:
secA1	MKKIMLVFITLILVSLPI AQQTEAKD	TEA'KD (SEQ ID NO:70)	*hly* (LLO)	*Listeria monocytogene s*	44
	MTDKKSENQTEKTETK ENKGMTRREMLKLSAV AGTGIAVGATGLGTILN VVDQVDKALT	DKA'LT (SEQ ID NO:71)	lmo0367	*Listeria monocytogene s*	45
	MAYDSRFDEWVQKLK EESFQNNTFDRRKFIQG AGKIAGLSLGLTIAQSV GAFG	VGA'FG (SEQ ID NO:72)	PhoD (alkaline phosphata se)	*Bacillus subtillis*	46

[00219] It should be appreciated by those skilled in the art that there exists a variety of proteins secreted via the Tat pathway among diverse bacterial genera, and that selected

Tat signal peptides corresponding to these proteins can be fused genetically in-frame to a desired sequence encoding a heterologous protein, to facilitate secretion of the functionally linked Tat signal peptide-heterologous protein chimera via the Tat pathway. Provided below are non-limiting examples of proteins from *Bacillus subtilis* and *Listeria* (*innocua* and *monocytogenes*) that are predicted to utilize Tat pathway secretion.

Putative *Bacillus subtilis* Proteins Secreted by Tat Pathwayhttp://www.sas.upenn.edu/~pohlschr/TABLE1.html

[00220] >gi|2635523|emb|CAB15017.1| similar to two (component sensor histidine kinase (YtsA) (Bacillus subtilis)

[00221] >gi|2632548|emb|CAB12056.1| phosphodiesterase/alkaline phosphatase D (Bacillus subtilis)

[00222] >gi|2632573|emb|CAB12081.1| similar to hypothetical proteins (Bacillus subtilis)

[00223] >gi|2633776|emb|CAB13278.1| similar to hypothetical proteins (Bacillus subtilis)

[00224] >gi|2634674|emb|CAB14172.1| menaquinol:cytochrome c oxidoreductase (iron (sulfur subunit) (Bacillus subtilis)

[00225] >gi|2635595|emb|CAB15089.1| yubF (Bacillus subtilis)

[00226] >gi|2636361|emb|CAB15852.1| alternate gene name: ipa (29d~similar to hypothetical proteins (Bacillus subtilis)

Putative *Listeria* Proteins Secreted by Tat Pathwayhttp://www.sas.upenn.edu/~pohlschr/TABLE1.html

[00227] *Listeria innocua*http://www.sas.upenn.edu/~pohlschr/TABLE1.html

[00228] >gi|16799463|ref|NP_469731.1| conserved hypothetical protein similar to B. subtilis YwbN protein (*Listeria* innocua)

[00229] >gi|16801368|ref|NP_471636.1| similar to 3 (oxoacyl (acyl (carrier protein synthase (*Listeria* innocua)

[00230] *Listeria monocytogenes EGD (e)* http://www.sas.upenn.edu/~pohlschr/TABLE1.html

[00231] >gi|16802412|ref|NP_463897.1| conserved hypothetical protein similar to B. subtilis YwbN protein (*Listeria* monocytogenes EGD (e)

[00232] Organisms utilize codon bias to regulate expression of particular endogenous genes. Thus, signal peptides utilized for secretion of selected heterologous proteins may not contain codons that utilize preferred codons, resulting in non-optimal levels of protein synthesis. In some some embodiments, the signal peptide sequence fused in frame with a

gene encoding a selected heterologous protein is codon-optimized for codon usage in a selected bacterium. In some embodiments for expression and secretion from recombinant *Listeria monocytogenes*, a nucleotide sequence of a selected signal peptide is codon optimized for expression in *Listeria monocytogenes*, according to the table (*ibid.*)

5.7.1.5. Transcription Termination Sequence

[00233] In some embodiments, a transcription termination sequence can be inserted into the heterologous protein expression cassette, downstream from the C-terminus of the translational stop codon related to the heterologous protein. Appropriate sequence elements known to those who are skilled in the art that promote either rho-dependent or rho-independent transcription termination can be placed in the heterologous protein expression cassette.

5.8 Anti-Idiotypic Antibodies

[00234] The present invention relates to methods and compositions utilizing EphA2 vaccines for eliciting immune responses against EphA2-expressing cells and treatment and prevention of disorders involving EphA2-expressing cells. In certain embodiments, the EphA2 vaccines of the invention comprises an anti-idiotype of an anti-EphA2 antibody.

[00235] The idiotopes on a single antibody molecule are thought to mimic and be the "internal image" of any foreign or self epitope at the molecular level. By means of Mab technology, an antibody against an EphA2 epitope is produced, purified and subsequently used as an immunogen to elicit an anti-idiotypic antibody which may be an internal image of the original EphA2 epitope. Thus, as predicted by the Jerne "network" theory (Jerne, 1974, *Ann. Inst. Pasteur. Immun.* 125C:373-389), immunization with an anti-idiotypic antibody that is directed against antigen combining sites of an anti-EphA2 epitope antibody would elicit a humoral immune response specific for the nominal antigen. The resulting anti-anti-idiotypic antibody should react with the original primary EphA2 epitope.

[00236] Thus, EphA2 antibodies can be utilized to generate anti-idiotype antibodies that using techniques well known to those skilled in the art. (*See, e.g.*, Greenspan & Bona, 1993, *FASEB* 17(5):437-444; and Nissinoff, 1991, *J. Immunol.* 147(8)2429-2438). For example, antibodies which bind to EphA2 can be used to generate anti-idiotypes that, when administered to a subject, can elicit a humoral immune response against EphA2. Such anti-idiotypes (including molecules comprising, or alternatively consisting of, antibody fragments or variants, such as Fab fragments of such anti-idiotypes) can be used in therapeutic regimens to elicit an immune response against hyperproliferative cells that

express EphA2 and thus be useful in treating, preventing or managing hyperproliferative diseases involving EphA2-overexpressing cells.

5.9 Prophylactic/Therapeutic Methods

[00237] The present invention encompasses methods for treating, preventing, or managing a disease or disorder associated with overexpression of EphA2 and/or cell hyperproliferative disorders, preferably cancer, in a subject comprising administering one or more EphA2 vaccines of the invention.

[00238] The present invention further encompasses methods for eliciting an immune response against an EphA2-expressing cell associated with a hyperproliferative disorder, comprising administering to a subject one or more EphA2 vaccines of the invention in an amount effective for eliciting an immune response against the EphA2-expressing cell.

[00239] An EphA2 vaccine may comprise one more EphA2 antigenic peptides, one ore more EphA2 antigenic peptide expression vehicles, or antigen presenting cells sensitized with one ore more EphA2 antigenic peptides.

[00240] In another specific embodiment, the disorder to be treated, prevented, or managed is a pre-cancerous condition associated with cells that overexpress EphA2. In more specific embodiments, the pre-cancerous condition is high-grade prostatic intraepithelial neoplasia (PIN), fibroadenoma of the breast, fibrocystic disease, or compound nevi.

[00241] In one embodiment, the peptides of the invention can be administered in combination with one or more other therapeutic agents useful in the treatment, prevention or management of diseases or disorders associated with EphA2 overexpression, hyperproliferative disorders, and/or cancer. In certain embodiments, one or more EphA2 antigenic peptides of the invention are administered to a mammal, preferably a human, concurrently with one or more other therapeutic agents useful for the treatment of cancer. The term "concurrently" is not limited to the administration of prophylactic or therapeutic agents at exactly the same time, but rather it is meant that the EphA2 antigenic peptides of the invention and the other agent are administered to a subject in a sequence and within a time interval such that the peptides of the invention can act together with the other agent to provide an increased benefit than if they were administered otherwise. For example, each prophylactic or therapeutic agent may be administered at the same time or sequentially in any order at different points in time; however, if not administered at the same time, they should be administered sufficiently close in time so as to provide the desired therapeutic or prophylactic effect. Each therapeutic agent can be administered separately, in any appropriate form and by any suitable route. In other embodiments, the EphA2 antigenic

peptides of the invention are administered before, concurrently or after surgery. Preferably the surgery completely removes localized tumors or reduces the size of large tumors. Surgery can also be done as a preventive measure or to relieve pain.

[00242] In various embodiments, the prophylactic or therapeutic agents are administered less than 1 hour apart, at about 1 hour apart, at about 1 hour to about 2 hours apart, at about 2 hours to about 3 hours apart, at about 3 hours to about 4 hours apart, at about 4 hours to about 5 hours apart, at about 5 hours to about 6 hours apart, at about 6 hours to about 7 hours apart, at about 7 hours to about 8 hours apart, at about 8 hours to about 9 hours apart, at about 9 hours to about 10 hours apart, at about 10 hours to about 11 hours apart, at about 11 hours to about 12 hours apart, no more than 24 hours apart or no more than 48 hours apart. In preferred embodiments, two or more components are administered within the same patient visit.

[00243] The dosage amounts and frequencies of administration provided herein are encompassed by the terms therapeutically effective and prophylactically effective. The dosage and frequency further will typically vary according to factors specific for each patient depending on the specific therapeutic or prophylactic agents administered, the severity and type of cancer, the route of administration, as well as age, body weight, response, and the past medical history of the patient. Suitable regimens can be selected by one skilled in the art by considering such factors and by following, for example, dosages reported in the literature and recommended in the *Physician's Desk Reference* (56th ed., 2002).

5.9.1 Patient Population

[00244] The invention provides methods for treating, preventing, and managing a disease or disorder associated with EphA2 overexpression and/or hyperproliferative cell disease, particularly cancer, by administrating to a subject in need thereof one or more EphA2 vaccines of the invention in a therapeutically or prophylactically effective amount or an amount effective to elicit an immune response against EphA2-expressing cells associated with the hyperproliferative disorder. In another embodiment, the EphA2 vaccines of the invention can be administered in combination with one or more other therapeutic or prophylactic agents. The subject is preferably a mammal such as non-primate (*e.g.*, cows, pigs, horses, cats, dogs, rats, *etc.*) and a primate (*e.g.*, monkey, such as a cynomolgous monkey and a human). In a preferred embodiment, the subject is a human.

[00245] Specific examples of cancers that can be treated by the methods encompassed by the invention include, but are not limited to, cancers that overexpress EphA2. In a further embodiment, the cancer is of an epithelial origin. Examples of such

cancers are cancer of the lung, colon, ovary, esophagus, prostate, breast, and skin. Other cancers include cancer of the bladder and pancreas and renal cell carcinoma and melanoma. In yet a further embodiment, the cancer is of a T cell origin. Examples of such cancers are leukemias and lymphomas. Additional cancers are listed by example and not by limitation in the following section 5.9.1.1. In particular embodiments, methods of the invention can be used to treat and/or prevent metastasis from primary tumors.

[00246] The methods and compositions of the invention comprise the administration of one or more EphA2 vaccines of the invention to subjects/patients suffering from or expected to suffer from cancer, *e.g.*, have a genetic predisposition for a particular type of cancer, have been exposed to a carcinogen, or are in remission from a particular cancer. As used herein, "cancer" refers to primary or metastatic cancers. Such patients may or may not have been previously treated for cancer. The methods and compositions of the invention may be used as a first line or second line cancer treatment. Included in the invention is also the treatment of patients undergoing other cancer therapies and the methods and compositions of the invention can be used before any adverse effects or intolerance of these other cancer therapies occurs. The invention also encompasses methods for administering one or more EphA2 vaccines of the invention to treat or ameliorate symptoms in refractory patients. In a certain embodiment, that a cancer is refractory to a therapy means that at least some significant portion of the cancer cells are not killed or their cell division arrested. The determination of whether the cancer cells are refractory can be made either *in vivo* or *in vitro* by any method known in the art for assaying the effectiveness of treatment on cancer cells, using the art-accepted meanings of "refractory" in such a context. In various embodiments, a cancer is refractory where the number of cancer cells has not been significantly reduced, or has increased. The invention also encompasses methods for administering one or more EphA2 vaccines to prevent the onset or recurrence of cancer in patients predisposed to having cancer.

[00247] In particular embodiments, the EphA2 vaccines of the invention are administered to reverse resistance or reduced sensitivity of cancer cells to certain hormonal, radiation and chemotherapeutic agents thereby resensitizing the cancer cells to one or more of these agents, which can then be administered (or continue to be administered) to treat or manage cancer, including to prevent metastasis. In a specific embodiment, the EphA2 vaccines of the invention are administered to patients with increased levels of the cytokine IL-6, which has been associated with the development of cancer cell resistance to different treatment regimens, such as chemotherapy and hormonal therapy. In another specific embodiment, the EphA2 vaccines of the invention are administered to patients suffering

from breast cancer that have a decreased responsiveness or are refractory to tamoxifen treatment. In another specific embodiment, the EphA2 vaccines of the invention are administered to patients with increased levels of the cytokine IL-6, which has been associated with the development of cancer cell resistance to different treatment regimens, such as chemotherapy and hormonal therapy.

[00248] In alternate embodiments, the invention provides methods for treating patients' cancer by administering one or more EphA2 vaccines of the invention in combination with any other treatment or to patients who have proven refractory to other treatments but are no longer on these treatments. In certain embodiments, the patients being treated by the methods of the invention are patients already being treated with chemotherapy, radiation therapy, hormonal therapy, or biological therapy/immunotherapy. Among these patients are refractory patients and those with cancer despite treatment with existing cancer therapies. In other embodiments, the patients have been treated and have no disease activity and one or more agonistic peptides of the invention are administered to prevent the recurrence of cancer.

[00249] In preferred embodiments, the existing treatment is chemotherapy. In particular embodiments, the existing treatment includes administration of chemotherapies including, but not limited to, methotrexate, taxol, mercaptopurine, thioguanine, hydroxyurea, cytarabine, cyclophosphamide, ifosfamide, nitrosoureas, cisplatin, carboplatin, mitomycin, dacarbazine, procarbizine, etoposides, campathecins, bleomycin, doxorubicin, idarubicin, daunorubicin, dactinomycin, plicamycin, mitoxantrone, asparaginase, vinblastine, vincristine, vinorelbine, paclitaxel, docetaxel, etc. Among these patients are patients treated with radiation therapy, hormonal therapy and/or biological therapy/immunotherapy. Also among these patients are those who have undergone surgery for the treatment of cancer.

[00250] Alternatively, the invention also encompasses methods for treating patients undergoing or having undergone radiation therapy. Among these are patients being treated or previously treated with chemotherapy, hormonal therapy and/or biological therapy/immunotherapy. Also among these patients are those who have undergone surgery for the treatment of cancer.

[00251] In other embodiments, the invention encompasses methods for treating patients undergoing or having undergone hormonal therapy and/or biological therapy/immunotherapy. Among these are patients being treated or having been treated with chemotherapy and/or radiation therapy. Also among these patients are those who have undergone surgery for the treatment of cancer.

[00252] Additionally, the invention also provides methods of treatment of cancer as an alternative to chemotherapy, radiation therapy, hormonal therapy, and/or biological therapy/immunotherapy where the therapy has proven or may prove too toxic, *i.e.*, results in unacceptable or unbearable side effects, for the subject being treated. The subject being treated with the methods of the invention may, optionally, be treated with other cancer treatments such as surgery, chemotherapy, radiation therapy, hormonal therapy or biological therapy, depending on which treatment was found to be unacceptable or unbearable.

[00253] In other embodiments, the invention provides administration of one or more EphA2 vaccines of the invention without any other cancer therapies for the treatment of cancer, but who have proved refractory to such treatments. In specific embodiments, patients refractory to other cancer therapies are administered one or more EphA2 vaccines in the absence of cancer therapies.

[00254] In other embodiments, patients with a pre-cancerous condition associated with cells that overexpress EphA2 can be administered vaccines of the invention to treat the disorder and decrease the likelihood that it will progress to malignant cancer. In a specific embodiments, the pre-cancerous condition is high-grade prostatic intraepithelial neoplasia (PIN), fibroadenoma of the breast, fibrocystic disease, or compound nevi.

[00255] In yet other embodiments, the invention provides methods of treating, preventing and managing non-cancer hyperproliferative cell disorders, particularly those associated with overexpression of EphA2, including but not limited to, asthma, chromic obstructive pulmonary disorder (COPD), restenosis (smooth muscle and/or endothelial), psoriasis, etc. These methods include methods analogous to those described above for treating, preventing and managing cancer, for example, by administering the EphA2 vaccines of the invention, combination therapy, administration to patients refractory to particular treatments, etc.

5.9.1.1. Cancers

[00256] Cancers and related disorders that can be treated, prevented, or managed by methods and compositions of the present invention include but are not limited to cancers of an epithelial cell origin. Examples of such cancers include the following: leukemias, such as but not limited to, acute leukemia, acute lymphocytic leukemia, acute myelocytic leukemias, such as, myeloblastic, promyelocytic, myelomonocytic, monocytic, and erythroleukemia leukemias and myelodysplastic syndrome; chronic leukemias, such as but not limited to, chronic myelocytic (granulocytic) leukemia, chronic lymphocytic leukemia, hairy cell leukemia; polycythemia vera; lymphomas such as but not limited to Hodgkin's disease, non-Hodgkin's disease; multiple myelomas such as but not limited to smoldering

multiple myeloma, nonsecretory myeloma, osteosclerotic myeloma, plasma cell leukemia, solitary plasmacytoma and extramedullary plasmacytoma; Waldenström's macroglobulinemia; monoclonal gammopathy of undetermined significance; benign monoclonal gammopathy; heavy chain disease; bone and connective tissue sarcomas such as but not limited to bone sarcoma, osteosarcoma, chondrosarcoma, Ewing's sarcoma, malignant giant cell tumor, fibrosarcoma of bone, chordoma, periosteal sarcoma, soft-tissue sarcomas, angiosarcoma (hemangiosarcoma), fibrosarcoma, Kaposi's sarcoma, leiomyosarcoma, liposarcoma, lymphangiosarcoma, neurilemmoma, rhabdomyosarcoma, synovial sarcoma; brain tumors such as but not limited to, glioma, astrocytoma, brain stem glioma, ependymoma, oligodendroglioma, nonglial tumor, acoustic neurinoma, craniopharyngioma, medulloblastoma, meningioma, pineocytoma, pineoblastoma, primary brain lymphoma; breast cancer including but not limited to ductal carcinoma, adenocarcinoma, lobular (small cell) carcinoma, intraductal carcinoma, medullary breast cancer, mucinous breast cancer, tubular breast cancer, papillary breast cancer, Paget's disease, and inflammatory breast cancer; adrenal cancer such as but not limited to pheochromocytom and adrenocortical carcinoma; thyroid cancer such as but not limited to papillary or follicular thyroid cancer, medullary thyroid cancer and anaplastic thyroid cancer; pancreatic cancer such as but not limited to, insulinoma, gastrinoma, glucagonoma, vipoma, somatostatin-secreting tumor, and carcinoid or islet cell tumor; pituitary cancers such as but limited to Cushing's disease, prolactin-secreting tumor, acromegaly, and diabetes insipius; eye cancers such as but not limited to ocular melanoma such as iris melanoma, choroidal melanoma, and cilliary body melanoma, and retinoblastoma; vaginal cancers such as squamous cell carcinoma, adenocarcinoma, and melanoma; vulvar cancer such as squamous cell carcinoma, melanoma, adenocarcinoma, basal cell carcinoma, sarcoma, and Paget's disease; cervical cancers such as but not limited to, squamous cell carcinoma, and adenocarcinoma; uterine cancers such as but not limited to endometrial carcinoma and uterine sarcoma; ovarian cancers such as but not limited to, ovarian epithelial carcinoma, borderline tumor, germ cell tumor, and stromal tumor; esophageal cancers such as but not limited to, squamous cancer, adenocarcinoma, adenoid cystic carcinoma, mucoepidermoid carcinoma, adenosquamous carcinoma, sarcoma, melanoma, plasmacytoma, verrucous carcinoma, and oat cell (small cell) carcinoma; stomach cancers such as but not limited to, adenocarcinoma, fungating (polypoid), ulcerating, superficial spreading, diffusely spreading, malignant lymphoma, liposarcoma, fibrosarcoma, and carcinosarcoma; colon cancers; rectal cancers; liver cancers such as but not limited to hepatocellular carcinoma and hepatoblastoma; gallbladder cancers such as adenocarcinoma;

cholangiocarcinomas such as but not limited to pappillary, nodular, and diffuse; lung cancers such as non-small cell lung cancer, squamous cell carcinoma (epidermoid carcinoma), adenocarcinoma, large-cell carcinoma and small-cell lung cancer; testicular cancers such as but not limited to germinal tumor, seminoma, anaplastic, classic (typical), spermatocytic, nonseminoma, embryonal carcinoma, teratoma carcinoma, choriocarcinoma (yolk-sac tumor), prostate cancers such as but not limited to, prostatic intraepithelial neoplasia, adenocarcinoma, leiomyosarcoma, and rhabdomyosarcoma; penal cancers; oral cancers such as but not limited to squamous cell carcinoma; basal cancers; salivary gland cancers such as but not limited to adenocarcinoma, mucoepidermoid carcinoma, and adenoidcystic carcinoma; pharynx cancers such as but not limited to squamous cell cancer, and verrucous; skin cancers such as but not limited to, basal cell carcinoma, squamous cell carcinoma and melanoma, superficial spreading melanoma, nodular melanoma, lentigo malignant melanoma, acral lentiginous melanoma; kidney cancers such as but not limited to renal cell carcinoma, adenocarcinoma, hypernephroma, fibrosarcoma, transitional cell cancer (renal pelvis and/ or uterer); Wilms' tumor; bladder cancers such as but not limited to transitional cell carcinoma, squamous cell cancer, adenocarcinoma, carcinosarcoma. In addition, cancers include myxosarcoma, osteogenic sarcoma, endotheliosarcoma, lymphangioendotheliosarcoma, mesothelioma, synovioma, hemangioblastoma, epithelial carcinoma, cystadenocarcinoma, bronchogenic carcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma and papillary adenocarcinomas (for a review of such disorders, see Fishman et al., 1985, *Medicine*, 2d Ed., J.B. Lippincott Co., Philadelphia and Murphy et al., 1997, *Informed Decisions: The Complete Book of Cancer Diagnosis, Treatment, and Recovery*, Viking Penguin, Penguin Books U.S.A., Inc., United States of America)

[00257] Accordingly, the methods and compositions of the invention are also useful in the treatment or prevention of a variety of cancers or other abnormal proliferative diseases, including (but not limited to) the following: carcinoma, including that of the bladder, breast, ovary, oesophagus, colon, kidney, liver, lung, ovary, pancreas, stomach, cervix, thyroid and skin; including squamous cell carcinoma; hematopoietic tumors of lymphoid lineage, including leukemia, acute lymphocytic leukemia, acute lymphoblastic leukemia, B-cell lymphoma, T-cell lymphoma, Burkitt's lymphoma; hematopoietic tumors of myeloid lineage, including acute and chronic myelogenous leukemias and promyelocytic leukemia; tumors of mesenchymal origin, including fibrosarcoma and rhabdomyoscarcoma; other tumors, including melanoma, seminoma, tetratocarcinoma, neuroblastoma and glioma; tumors of the central and peripheral nervous system, including astrocytoma,

neuroblastoma, glioma, and schwannomas; tumors of mesenchymal origin, including fibrosarcoma, rhabdomyoscarama, and osteosarcoma; and other tumors, including melanoma, xeroderma pigmentosum, keratoactanthoma, seminoma, thyroid follicular cancer and teratocarcinoma. It is also contemplated that cancers caused by aberrations in apoptosis would also be treated by the methods and compositions of the invention. Such cancers may include but not be limited to follicular lymphomas, carcinomas with p53 mutations, hormone dependent tumors of the breast, prostate and ovary, and precancerous lesions such as familial adenomatous polyposis, and myelodysplastic syndromes. In specific embodiments, malignancy or dysproliferative changes (such as metaplasias and dysplasias), or hyperproliferative disorders, are treated or prevented in the skin, lung, colon, breast, prostate, bladder, kidney, pancreas, ovary, or uterus. In other specific embodiments, sarcoma, melanoma, or leukemia is treated or prevented.

[00258] In some embodiments, the cancer is malignant and overexpresses EphA2. In other embodiments, the disorder to be treated is a pre-cancerous condition associated with cells that overexpress EphA2. In a specific embodiments, the pre-cancerous condition is high-grade prostatic intraepithelial neoplasia (PIN), fibroadenoma of the breast, fibrocystic disease, or compound nevi.

[00259] In preferred embodiments, the methods and compositions of the invention are used for the treatment and/or prevention of breast, colon, ovarian, oesophageal, lung, and prostate cancers and melanoma and are provided below by example rather than by limitation.

[00260] In another preferred embodiment, the methods and compositions of the invention are used for the treatment and/or prevention of cancers of T cell origin, including, but not limited to, leukemias and lymphomas.

5.9.1.2. Treatment of Breast Cancer

[00261] In specific embodiments, patients with breast cancer are administered an effective amount of one or more EphA2 vaccines of the invention. In another embodiment, the peptides of the invention can be administered in combination with an effective amount of one or more other agents useful for breast cancer therapy including but not limited to: doxorubicin, epirubicin, the combination of doxorubicin and cyclophosphamide (AC), the combination of cyclophosphamide, doxorubicin and 5-fluorouracil (CAF), the combination of cyclophosphamide, epirubicin and 5-fluorouracil (CEF), herceptin, tamoxifen, the combination of tamoxifen and cytotoxic chemotherapy, taxanes (such as docetaxel and paclitaxel). In a further embodiment, peptides of the invention can be administered with

taxanes plus standard doxorubicin and cyclophosphamide for adjuvant treatment of node-positive, localized breast cancer.

[00262] In a specific embodiment, patients with pre-cancerous fibroadenoma of the breast or fibrocystic disease are administered an EphA2 vaccine of the invention to treat the disorder and decrease the likelihood that it will progress to malignant breast cancer. In another specific embodiment, patients refractory to treatment, particularly hormonal therapy, more particularly tamoxifen therapy, are administered an EphA2 vaccine of the invention to treat the cancer and/or render the patient non-refractory or responsive.

5.9.1.3. Treatment of Colon Cancer

[00263] In specific embodiments, patients with colon cancer are administered an effective amount of one or more EphA2 vaccines of the invention. In another embodiment, the peptides of the invention can be administered in combination with an effective amount of one or more other agents useful for colon cancer therapy including but not limited to: the combination of 5-FU and leucovorin, the combination of 5-FU and levamisole, irinotecan (CPT-11) or the combination of irinotecan, 5-FU and leucovorin (IFL).

5.9.1.4. Treatment of Prostate Cancer

[00264] In specific embodiments, patients with prostate cancer are administered an effective amount of one or more EphA2 vaccines of the invention. In another embodiment, the peptides of the invention can be administered in combination with an effective amount of one or more other agents useful for prostate cancer therapy including but not limited to: external-beam radiation therapy, interstitial implantation of radioisotopes (*i.e.*, I^{125}, palladium, iridium), leuprolide or other LHRH agonists, non-steroidal antiandrogens (flutamide, nilutamide, bicalutamide), steroidal antiandrogens (cyproterone acetate), the combination of leuprolide and flutamide, estrogens such as DES, chlorotrianisene, ethinyl estradiol, conjugated estrogens U.S.P., DES-diphosphate, radioisotopes, such as strontium-89, the combination of external-beam radiation therapy and strontium-89, second-line hormonal therapies such as aminoglutethimide, hydrocortisone, flutamide withdrawal, progesterone, and ketoconazole, low-dose prednisone, or other chemotherapy regimens reported to produce subjective improvement in symptoms and reduction in PSA level including docetaxel, paclitaxel, estramustine/docetaxel, estramustine/etoposide, estramustine/vinblastine, and estramustine/paclitaxel.

[00265] In a specific embodiment, patients with pre-cancerous high-grade prostatic intraepithelial neoplasia (PIN) are administered an EphA2 vaccine of the invention to treat the disorder and decrease the likelihood that it will progress to malignant prostate cancer.

5.9.1.5. Treatment of Melanoma

[00266] In specific embodiments, patients with melanoma are administered an effective amount of one or more EphA2 vaccines of the invention. In another embodiment, the peptides of the invention can be administered in combination with an effective amount of one or more other agents useful for melanoma cancer therapy including but not limited to: dacarbazine (DTIC), nitrosoureas such as carmustine (BCNU) and lomustine (CCNU), agents with modest single agent activity including vinca alkaloids, platinum compounds, and taxanes, the Dartmouth regimen (cisplatin, BCNU, and DTIC), interferon alpha (IFN-A), and interleukin-2 (IL-2). In a specific embodiment, an effective amount of one or more EphA2 vaccines of the invention can be administered in combination with isolated hyperthermic limb perfusion (ILP) with melphalan (L-PAM), with or without tumor necrosis factor-alpha (TNF-alpha) to patients with multiple brain metastases, bone metastases, and spinal cord compression to achieve symptom relief and some shrinkage of the tumor with radiation therapy.

[00267] In a specific embodiment, patients with pre-cancerous compound nevi are administered an EphA2 vaccine of the invention to treat the disorder and decrease the likelihood that it will progress to malignant melanoma.

5.9.1.6. Treatment of Ovarian Cancer

[00268] In specific embodiments, patients with ovarian cancer are administered an effective amount of one or more EphA2 vaccines of the invention. In another embodiment, the peptides of the invention can be administered in combination with an effective amount of one or more other agents useful for ovarian cancer therapy including but not limited to: intraperitoneal radiation therapy, such as P^{32} therapy, total abdominal and pelvic radiation therapy, cisplatin, the combination of paclitaxel (Taxol) or docetaxel (Taxotere) and cisplatin or carboplatin, the combination of cyclophosphamide and cisplatin, the combination of cyclophosphamide and carboplatin, the combination of 5-FU and leucovorin, etoposide, liposomal doxorubicin, gemcitabine or topotecan. It is contemplated that an effective amount of one or more EphA2 vaccines of the invention is administered in combination with the administration Taxol for patients with platinum-refractory disease. Included is the treatment of patients with refractory ovarian cancer including administration of: ifosfamide in patients with disease that is platinum-refractory, hexamethylmelamine (HMM) as salvage chemotherapy after failure of cisplatin-based combination regimens, and tamoxifen in patients with detectable levels of cytoplasmic estrogen receptor on their tumors.

5.9.1.7. Treatment of Lung Cancers

[00269] In specific embodiments, patients with small lung cell cancer are administered an effective amount of one or more EphA2 vaccines of the invention. In another embodiment, the peptides of the invention can be administered in combination with an effective amount of one or more other agents useful for lung cancer therapy including but not limited to: thoracic radiation therapy, cisplatin, vincristine, doxorubicin, and etoposide, alone or in combination, the combination of cyclophosphamide, doxorubicin, vincristine/etoposide, and cisplatin (CAV/EP), local palliation with endobronchial laser therapy, endobronchial stents, and/or brachytherapy.

[00270] In other specific embodiments, patients with non-small lung cell cancer are administered an effective amount of one or more EphA2 vaccines of the invention in combination with an effective amount of one or more other agents useful for lung cancer therapy including but not limited to: palliative radiation therapy, the combination of cisplatin, vinblastine and mitomycin, the combination of cisplatin and vinorelbine, paclitaxel, docetaxel or gemcitabine, the combination of carboplatin and paclitaxel, interstitial radiation therapy for endobronchial lesions or stereotactic radiosurgery.

5.9.1.8. Treatment of T Cell Malignancies

[00271] In specific embodiments, patients with T cell malignancies, such as leukemias and lymphomas (see, e.g., section 5.9.1.1), are administered an effective amount of one or more EphA2 vaccines of the invention. In another embodiment, the EphA2 vaccines of the invention can be administered in combination with an effective amount of one or more other agents useful for the prevention, treatment or amelioration of cancer, particularly T cell malignancies or one or more symptoms thereof, said combination therapies comprising administering to a subject in need thereof a prophylactically or therapeutically effective amount of one or more EphA2 vaccines of the invention and a prophylactically or therapeutically effective amount of one or more cancer therapies, including chemotherapies, hormonal therapies, biological therapies, immunotherapies, or radiation therapies.

[00272] In another specific embodiment, patients with T cell malignancies are administered an effective amount of one or more EphA2 vaccines of the invention in combination with one or more cancer chemotherapeutic agents, such as but not limited to: doxorubicin, epirubicin, cyclophosphamide, 5-fluorouracil, taxanes such as docetaxel and paclitaxel, leucovorin, levamisole, irinotecan, estramustine, etoposide, vinblastine, dacarbazine, nitrosoureas such as carmustine and lomustine, vinca alkaloids, platinum compounds, cisplatin, mitomycin, vinorelbine, gemcitabine, carboplatin,

hexamethylmelamine and/or topotecan. Such methods can optionally further comprise the administration of other cancer therapies, such as but not limited to radiation therapy, biological therapies, hormonal therapies and/or surgery.

[00273] In yet another specific embodiment, patients with T cell malignancies are administered an effective amount of one or more EphA2 vaccines of the invention in combination with one or more types of radiation therapy, such as external-beam radiation therapy, interstitial implantation of radioisotopes (I-125, palladium, iridium), radioisotopes such as strontium-89, thoracic radiation therapy, intraperitoneal P-32 radiation therapy, and/or total abdominal and pelvic radiation therapy. Such methods can optionally further comprise the administration of other cancer therapies, such as but not limited to chemotherapies, biological therapies/immunotherapies, hormonal therapies and/or surgery.

[00274] In yet another specific embodiment, patients with T cell malignancies are administered an effective amount of one or more EphA2 vaccines of the invention in combination with one or more biological therapies/immunotherapies or hormonal therapies, such as tamoxifen, leuprolide or other LHRH agonists, non-steroidal antiandrogens (flutamide, nilutamide, bicalutamide), steroidal antiandrogens (cyproterone acetate), estrogens (DES, chlorotrianisene, ethinyl estradiol, conjugated estrogens U.S.P., DES-diphosphate), aminoglutethimide, hydrocortisone, flutamide withdrawal, progesterone, ketoconazole, prednisone, interferon-α, interleukin-2, tumor necrosis factor-α, and/or melphalan. Biological therapies also included are cytokines such as but not limited to TNF ligand family members such as TRAIL anti-cancer agonists that induce apoptosis, TRAIL antibodies that bind to TRAIL receptors 1 and 2 otherwise known as DR4 and DR5 (Death Domain Containing Receptors 4 and 5), as well as DR4 and DR5. TRAIL and TRAIL antibodies, ligands and receptors are known in the art and described in U.S. Patent Nos. 6,342,363, 6,284,236, 6,072,047 and 5,763,223. Such methods can optionally further comprise the administration of other cancer therapies, such as but not limited to radiation therapy, chemotherapies, and/or surgery.

[00275] In yet another specific embodiment, patients with T cell malignancies are administered an effective amount of one or more EphA2 vaccines of the invention in combination with standard and experimental therapies of T cell malignancies. Standard and experimental therapies of T cell malignancies that can be used in the methods and compositions of the invention include, but are not limited to, antibody therapy (e.g., Campath®, anti-Tac, HuM291 (humanized murine IgG2 monoclonal antibody against CD3), antibody drug conjugates (e.g., Mylotarg), radiolabeled monoclonal antibodies (e.g., Bexxar, Zevalin, Lym-1)), cytokine therapy, aggressive combination chemotherapy with or

without cytotoxic agents, purine analogs, hematopoietic stem cell transplantation, and T cell mediated therapy (e.g., CD8+ T cells with anti-leukemic activity against target antigens including but not limited to leukemia specific proteins (e.g., bcr/abl, PML/RARa, EMV/AML-1), leukemia-associated proteins (e.g., proteinase 3, WT-1, h-TERT, hdm-2)). (See Riddell et el., 2002, Cancer Control, 9(2): 114-122; Dearden et al. , 2002, Medical Oncology, 19, Suppl. S27-32; Waldmann et al. 2000, Hemtaology (Am Soc Hematol Educ Program):394 408).

5.9.2 Other Prophylactic/Therapeutic Agents

[00276] In some embodiments, therapy by administration of one or more EphA2 vaccines is combined with the administration of one or more therapies such as, but not limited to, chemotherapies, radiation therapies, hormonal therapies, and/or biological therapies/immunotherapies. Prophylactic/therapeutic agents include, but are not limited to, proteinaceous molecules, including, but not limited to, peptides, polypeptides, proteins, including post-translationally modified proteins, peptides etc.; or small molecules (less than 1000 daltons), inorganic or organic compounds; or nucleic acid molecules including, but not limited to, double-stranded or single-stranded DNA, or double-stranded or single-stranded RNA, as well as triple helix nucleic acid molecules. Prophylactic/therapeutic agents can be derived from any known organism (including, but not limited to, animals, plants, bacteria, fungi, and protista, or viruses) or from a library of synthetic molecules.

[00277] In a specific embodiment, the methods of the invention encompass administration of an EphA2 vaccine of the invention in combination with the administration of one or more prophylactic/therapeutic agents that are inhibitors of kinases such as, but not limited to, ABL, ACK, AFK, AKT (*e.g.*, AKT-1, AKT-2, and AKT-3), ALK, AMP-PK, ATM, Auroral, Aurora2, bARK1, bArk2, BLK, BMX, BTK, CAK, CaM kinase, CDC2, CDK, CK, COT, CTD, DNA-PK, EGF-R, ErbB-1, ErbB-2, ErbB-3, ErbB-4, ERK (*e.g.*, ERK1, ERK2, ERK3, ERK4, ERK5, ERK6, ERK7), ERT-PK, FAK, FGR (*e.g.*, FGF1R, FGF2R), FLT (*e.g.*, FLT-1, FLT-2, FLT-3, FLT-4), FRK, FYN, GSK (*e.g.*, GSK1, GSK2, GSK3-alpha, GSK3-beta, GSK4, GSK5), G-protein coupled receptor kinases (GRKs), HCK, HER2, HKII, JAK (*e.g.*, JAK1, JAK2, JAK3, JAK4), JNK (*e.g.*, JNK1, JNK2, JNK3), KDR, KIT, IGF-1 receptor, IKK-1, IKK-2, INSR (insulin receptor), IRAK1, IRAK2, IRK, ITK, LCK, LOK, LYN, MAPK, MAPKAPK-1, MAPKAPK-2, MEK, MET, MFPK, MHCK, MLCK, MLK3, NEU, NIK, PDGF receptor alpha, PDGF receptor beta, PHK, PI-3 kinase, PKA, PKB, PKC, PKG, PRK1, PYK2, p38 kinases, p135tyk2, p34cdc2, p42cdc2, p42mapk, p44mpk, RAF, RET, RIP, RIP-2, RK, RON, RS kinase, SRC, SYK, S6K, TAK1, TEC, TIE1, TIE2, TRKA, TXK, TYK2, UL13, VEGFR1, VEGFR2, YES,

YRK, ZAP-70, and all subtypes of these kinases (see *e.g.*, Hardie and Hanks (1995) The Protein Kinase Facts Book, I and II, Academic Press, San Diego, Calif.). In preferred embodiments, an EphA2 vaccine of the invention is administered in combination with the administration of one or more prophylactic/therapeutic agents that are inhibitors of Eph receptor kinases (*e.g.*, EphA2, EphA4). In a most preferred embodiment, an EphA2 vaccine of the invention is administered in combination with the administration of one or more prophylactic/therapeutic agents that are inhibitors of EphA2.

[00278] In another specific embodiment, the methods of the invention encompass administration of an EphA2 vaccine of the invention in combination with the administration of one or more prophylactic/therapeutic agents that are angiogenesis inhibitors such as, but not limited to: Angiostatin (plasminogen fragment); antiangiogenic antithrombin III; Angiozyme; ABT-627; Bay 12-9566; Benefin; Bevacizumab; BMS-275291; cartilage-derived inhibitor (CDI); CAI; CD59 complement fragment; CEP-7055; Col 3; Combretastatin A-4; Endostatin (collagen XVIII fragment); fibronectin fragment; Gro-beta; Halofuginone; Heparinases; Heparin hexasaccharide fragment; HMV833; Human chorionic gonadotropin (hCG); IM-862; Interferon alpha/beta/gamma; Interferon inducible protein (IP-10); Interleukin-12; Kringle 5 (plasminogen fragment); Marimastat; Metalloproteinase inhibitors (TIMPs); 2-Methoxyestradiol; MMI 270 (CGS 27023A); MoAb IMC-1C11; Neovastat; NM-3; Panzem; PI-88; Placental ribonuclease inhibitor; Plasminogen activator inhibitor; Platelet factor-4 (PF4); Prinomastat; Prolactin 16kD fragment; Proliferin-related protein (PRP); PTK 787/ZK 222594; Retinoids; Solimastat; Squalamine; SS 3304; SU 5416; SU6668; SU11248; Tetrahydrocortisol-S; tetrathiomolybdate; thalidomide; Thrombospondin-1 (TSP-1); TNP-470; Transforming growth factor-beta (TGF-β); Vasculostatin; Vasostatin (calreticulin fragment); ZD6126; ZD6474; farnesyl transferase inhibitors (FTI); and bisphosphonates.

[00279] In another specific embodiment, the methods of the invention encompass administration of an EphA2 vaccine of the invention in combination with the administration of one or more prophylactic/therapeutic agents that are anti-cancer agents such as, but not limited to: acivicin, aclarubicin, acodazole hydrochloride, acronine, adozelesin, aldesleukin, altretamine, ambomycin, ametantrone acetate, aminoglutethimide, amsacrine, anastrozole, anthramycin, asparaginase, asperlin, azacitidine, azetepa, azotomycin, batimastat, benzodepa, bicalutamide, bisantrene hydrochloride, bisnafide dimesylate, bizelesin, bleomycin sulfate, brequinar sodium, bropirimine, busulfan, cactinomycin, calusterone, caracemide, carbetimer, carboplatin, carmustine, carubicin hydrochloride, carzelesin, cedefingol, chlorambucil, cirolemycin, cisplatin, cladribine, crisnatol mesylate,

cyclophosphamide, cytarabine, dacarbazine, dactinomycin, daunorubicin hydrochloride, decarbazine, decitabine, dexormaplatin, dezaguanine, dezaguanine mesylate, diaziquone, docetaxel, doxorubicin, doxorubicin hydrochloride, droloxifene, droloxifene citrate, dromostanolone propionate, duazomycin, edatrexate, eflornithine hydrochloride, elsamitrucin, enloplatin, enpromate, epipropidine, epirubicin hydrochloride, erbulozole, esorubicin hydrochloride, estramustine, estramustine phosphate sodium, etanidazole, etoposide, etoposide phosphate, etoprine, fadrozole hydrochloride, fazarabine, fenretinide, floxuridine, fludarabine phosphate, fluorouracil, flurocitabine, fosquidone, fostriecin sodium, gemcitabine, gemcitabine hydrochloride, hydroxyurea, idarubicin hydrochloride, ifosfamide, ilmofosine, interleukin 2 (including recombinant interleukin 2, or rIL2), interferon alpha-2a, interferon alpha-2b, interferon alpha-n1, interferon alpha-n3, interferon beta-I a, interferon gamma-I b, iproplatin, irinotecan hydrochloride, lanreotide acetate, letrozole, leuprolide acetate, liarozole hydrochloride, lometrexol sodium, lomustine, losoxantrone hydrochloride, masoprocol, maytansine, mechlorethamine hydrochloride, megestrol acetate, melengestrol acetate, melphalan, menogaril, mercaptopurine, methotrexate, methotrexate sodium, metoprine, meturedepa, mitindomide, mitocarcin, mitocromin, mitogillin, mitomalcin, mitomycin, mitosper, mitotane, mitoxantrone hydrochloride, mycophenolic acid, nitrosoureas, nocodazole, nogalamycin, ormaplatin, oxisuran, paclitaxel, pegaspargase, peliomycin, pentamustine, peplomycin sulfate, perfosfamide, pipobroman, piposulfan, piroxantrone hydrochloride, plicamycin, plomestane, porfimer sodium, porfiromycin, prednimustine, procarbazine hydrochloride, puromycin, puromycin hydrochloride, pyrazofurin, riboprine, rogletimide, safingol, safingol hydrochloride, semustine, simtrazene, sparfosate sodium, sparsomycin, spirogermanium hydrochloride, spiromustine, spiroplatin, streptonigrin, streptozocin, sulofenur, talisomycin, tecogalan sodium, tegafur, teloxantrone hydrochloride, temoporfin, teniposide, teroxirone, testolactone, thiamiprine, thioguanine, thiotepa, tiazofurin, tirapazamine, toremifene citrate, trestolone acetate, triciribine phosphate, trimetrexate, trimetrexate glucuronate, triptorelin, tubulozole hydrochloride, uracil mustard, uredepa, vapreotide, verteporfin, vinblastine sulfate, vincristine sulfate, vindesine, vindesine sulfate, vinepidine sulfate, vinglycinate sulfate, vinleurosine sulfate, vinorelbine tartrate, vinrosidine sulfate, vinzolidine sulfate, vorozole, zeniplatin, zinostatin, zorubicin hydrochloride. Other anti-cancer drugs include, but are not limited to: 20-epi-1,25 dihydroxyvitamin D3, 5-ethynyluracil, abiraterone, aclarubicin, acylfulvene, adecypenol, adozelesin, aldesleukin, ALL-TK antagonists, altretamine, ambamustine, amidox, amifostine, aminolevulinic acid, amrubicin, amsacrine, anagrelide, anastrozole, andrographolide, angiogenesis inhibitors, antagonist D, antagonist

G, antarelix, anti-dorsalizing morphogenetic protein-1, antiandrogens, antiestrogens, antineoplaston, aphidicolin glycinate, apoptosis gene modulators, apoptosis regulators, apurinic acid, ara-CDP-DL-PTBA, arginine deaminase, asulacrine, atamestane, atrimustine, axinastatin 1, axinastatin 2, axinastatin 3, azasetron, azatoxin, azatyrosine, baccatin III derivatives, balanol, batimastat, BCR/ABL antagonists, benzochlorins, benzoylstaurosporine, beta lactam derivatives, beta-alethine, betaclamycin B, betulinic acid, bFGF inhibitor, bicalutamide, bisantrene, bisaziridinylspermine, bisnafide, bistratene A, bizelesin, breflate, bropirimine, budotitane, buthionine sulfoximine, calcipotriol, calphostin C, camptothecin derivatives, canarypox IL-2, capecitabine, carboxamide-amino-triazole, carboxyamidotriazole, CaRest M3, CARN 700, cartilage derived inhibitor, carzelesin, casein kinase inhibitors (ICOS), castanospermine, cecropin B, cetrorelix, chloroquinoxaline sulfonamide, cicaprost, cis-porphyrin, cladribine, clomifene analogues, clotrimazole, collismycin A, collismycin B, combretastatin A4, combretastatin analogue, conagenin, crambescidin 816, crisnatol, cryptophycin 8, cryptophycin A derivatives, curacin A, cyclopentanthraquinones, cycloplatam, cypemycin, cytarabine ocfosfate, cytolytic factor, cytostatin, dacliximab, decitabine, dehydrodidemnin B, deslorelin, dexamethasone, dexifosfamide, dexrazoxane, dexverapamil, diaziquone, didemnin B, didox, diethylnorspermine, dihydro-5-azacytidine, dihydrotaxol, dioxamycin, diphenyl spiromustine, docetaxel, docosanol, dolasetron, doxifluridine, droloxifene, dronabinol, duocarmycin SA, ebselen, ecomustine, edelfosine, edrecolomab, eflornithine, elemene, emitefur, epirubicin, episteride, estramustine analogue, estrogen agonists, estrogen antagonists, etanidazole, etoposide phosphate, exemestane, fadrozole, fazarabine, fenretinide, filgrastim, finasteride, flavopiridol, flezelastine, fluasterone, fludarabine, fluorodaunorunicin hydrochloride, forfenimex, formestane, fostriecin, fotemustine, gadolinium texaphyrin, gallium nitrate, galocitabine, ganirelix, gelatinase inhibitors, gemcitabine, glutathione inhibitors, hepsulfam, heregulin, hexamethylene bisacetamide, hypericin, ibandronic acid, idarubicin, idoxifene, idramantone, ilmofosine, ilomastat, imidazoacridones, imiquimod, immunostimulant peptides, insulin-like growth factor-1 receptor inhibitor, interferon agonists, interferons, interleukins, iobenguane, iododoxorubicin, ipomeanol, iroplact, irsogladine, isobengazole, isohomohalicondrin B, itasetron, jasplakinolide, kahalalide F, lamellarin-N triacetate, lanreotide, leinamycin, lenograstim, lentinan sulfate, leptolstatin, letrozole, leukemia inhibiting factor, leukocyte alpha interferon, leuprolide+estrogen+progesterone, leuprorelin, levamisole, liarozole, linear polyamine analogue, lipophilic disaccharide peptide, lipophilic platinum compounds, lissoclinamide 7, lobaplatin, lombricine, lometrexol, lonidamine, losoxantrone, lovastatin,

loxoribine, lurtotecan, lutetium texaphyrin, lysofylline, lytic peptides, maitansine, mannostatin A, marimastat, masoprocol, maspin, matrilysin inhibitors, matrix metalloproteinase inhibitors, menogaril, merbarone, meterelin, methioninase, metoclopramide, MIF inhibitor, mifepristone, miltefosine, mirimostim, mismatched double stranded RNA, mitoguazone, mitolactol, mitomycin analogues, mitonafide, mitotoxin fibroblast growth factor-saporin, mitoxantrone, mofarotene, molgramostim, EphA2 vaccine, human chorionic gonadotrophin, monophosphoryl lipid A+myobacterium cell wall sk, mopidamol, multiple drug resistance gene inhibitor, multiple tumor suppressor 1-based therapy, mustard anticancer agent, mycaperoxide B, mycobacterial cell wall extract, myriaporone, N-acetyldinaline, N-substituted benzamides, nafarelin, nagrestip, naloxone+pentazocine, napavin, naphterpin, nartograstim, nedaplatin, nemorubicin, neridronic acid, neutral endopeptidase, nilutamide, nisamycin, nitric oxide modulators, nitroxide antioxidant, nitrullyn, O6-benzylguanine, octreotide, okicenone, oligonucleotides, onapristone, ondansetron, ondansetron, oracin, oral cytokine inducer, ormaplatin, osaterone, oxaliplatin, oxaunomycin, paclitaxel, paclitaxel analogues, paclitaxel derivatives, palauamine, palmitoylrhizoxin, pamidronic acid, panaxytriol, panomifene, parabactin, pazelliptine, pegaspargase, peldesine, pentosan polysulfate sodium, pentostatin, pentrozole, perflubron, perfosfamide, perillyl alcohol, phenazinomycin, phenylacetate, phosphatase inhibitors, picibanil, pilocarpine hydrochloride, pirarubicin, piritrexim, placetin A, placetin B, plasminogen activator inhibitor, platinum complex, platinum compounds, platinum-triamine complex, porfimer sodium, porfiromycin, prednisone, propyl bis-acridone, prostaglandin J2, proteasome inhibitors, protein A-based immune modulator, protein kinase C inhibitor, protein kinase C inhibitors, microalgal, protein tyrosine phosphatase inhibitors, purine nucleoside phosphorylase inhibitors, purpurins, pyrazoloacridine, pyridoxylated hemoglobin polyoxyethylene conjugate, raf antagonists, raltitrexed, ramosetron, ras farnesyl protein transferase inhibitors, ras inhibitors, ras-GAP inhibitor, retelliptine demethylated, rhenium Re 186 etidronate, rhizoxin, ribozymes, RII retinamide, rogletimide, rohitukine, romurtide, roquinimex, rubiginone B1, ruboxyl, safingol, saintopin, SarCNU, sarcophytol A, sargramostim, Sdi 1 mimetics, semustine, senescence derived inhibitor 1, sense oligonucleotides, signal transduction inhibitors, signal transduction modulators, single chain antigen binding protein, sizofiran, sobuzoxane, sodium borocaptate, sodium phenylacetate, solverol, somatomedin binding protein, sonermin, sparfosic acid, spicamycin D, spiromustine, splenopentin, spongistatin 1, squalamine, stem cell inhibitor, stem-cell division inhibitors, stipiamide, stromelysin inhibitors, sulfinosine, superactive vasoactive intestinal peptide antagonist, suradista, suramin, swainsonine, synthetic

glycosaminoglycans, tallimustine, tamoxifen methiodide, tauromustine, taxol, tazarotene, tecogalan sodium, tegafur, tellurapyrylium, telomerase inhibitors, temoporfin, temozolomide, teniposide, tetrachlorodecaoxide, tetrazomine, thaliblastine, thalidomide, thiocoraline, thioguanine, thrombopoietin, thrombopoietin mimetic, thymalfasin, thymopoietin receptor agonist, thymotrinan, thyroid stimulating hormone, tin ethyl etiopurpurin, tirapazamine, titanocene bichloride, topsentin, toremifene, totipotent stem cell factor, translation inhibitors, tretinoin, triacetyluridine, triciribine, trimetrexate, triptorelin, tropisetron, turosteride, tyrosine kinase inhibitors, tyrphostins, UBC inhibitors, ubenimex, urogenital sinus-derived growth inhibitory factor, urokinase receptor antagonists, vapreotide, variolin B, vector system, erythrocyte gene therapy, velaresol, veramine, verdins, verteporfin, vinorelbine, vinxaltine, vitaxin, vorozole, zanoterone, zeniplatin, zilascorb, and zinostatin stimalamer. Preferred additional anti-cancer drugs are 5-fluorouracil and leucovorin.

[00280] In more particular embodiments, the present invention also comprises the administration of one or more EphA2 vaccines of the invention in combination with the administration of one or more therapies such as, but not limited to anti-cancer agents such as those disclosed in Table 2, preferably for the treatment of breast, ovary, melanoma, prostate, colon and lung cancers as described above.

TABLE 2

Therapeutic Agent	Administration	Dose	Intervals
doxorubicin hydrochloride (Adriamycin RDF® and Adriamycin PFS®)	Intravenous	60-75 mg/m^2 on Day 1	21 day intervals
epirubicin hydrochloride (Ellence™)	Intravenous	100-120 mg/m^2 on Day 1 of each cycle or divided equally and given on Days 1-8 of the cycle	3-4 week cycles
fluorousacil	Intravenous	How supplied: 5 ml and 10 ml vials (containing 250 and 500 mg flourouracil respectively)	
docetaxel (Taxotere®)	Intravenous	60- 100 mg/m^2 over 1 hour	Once every 3 weeks
paclitaxel (Taxol®)	Intravenous	175 mg/m^2 over 3 hours	Every 3 weeks for 4 courses (administered sequentially to doxorubicin-containing combination chemotherapy)
tamoxifen citrate (Nolvadex®)	Oral (tablet)	20-40 mg Dosages greater than 20 mg should be given in divided doses (morning and evening)	Daily

Therapeutic Agent	Administration	Dose	Intervals
leucovorin calcium for injection	Intravenous or intramuscular injection	How supplied: 350 mg vial	Dosage is unclear from text. PDR 3610
luprolide acetate (Lupron®)	Single subcutaneous injection	1 mg (0.2 ml or 20 unit mark)	Once a day
flutamide (Eulexin®)	Oral (capsule)	250 mg (capsules contain 125 mg flutamide each)	3 times a day at 8 hour intervals (total daily dosage 750 mg)
nilutamide (Nilandron®)	Oral (tablet)	300 mg or 150 mg (tablets contain 50 or 150 mg nilutamide each)	300 mg once a day for 30 days followed by 150 mg once a day
bicalutamide (Casodex®)	Oral (tablet)	50 mg (tablets contain 50 mg bicalutamide each)	Once a day
progesterone	Injection	USP in sesame oil 50 mg/ml	
ketoconazole (Nizoral®)	Cream	2% cream applied once or twice daily depending on symptoms	
prednisone	Oral (tablet)	Initial dosage may vary from 5 mg to 60 mg per day depending on the specific disease entity being treated.	
estramustine phosphate sodium (Emcyt®)	Oral (capsule)	14 mg/ kg of body weight (*i.e.* one 140 mg capsule for each 10 kg or 22 lb of body weight)	Daily given in 3 or 4 divided doses
etoposide or VP-16	Intravenous	5 ml of 20 mg/ ml solution (100 mg)	
dacarbazine (DTIC-Dome®)	Intravenous	2-4.5 mg/knowing	Once a day for 10 days. May be repeated at 4 week intervals
polifeprosan 20 with carmustine implant (BCNU) (nitrosourea) (Gliadel®)	wafer placed in resection cavity	8 wafers, each containing 7.7 mg of carmustine, for a total of 61.6 mg, if size and shape of resection cavity allows	
cisplatin	Injection	How supplied: solution of 1 mg/ml in multi-dose vials of 50mL and 100mL	
mitomycin	Injection	supplied in 5 mg and 20 mg vials (containing 5 mg and 20 mg mitomycin)	
gemcitabine HCl (Gemzar®)	Intravenous	For NSCLC- 2 schedules have been investigated and the optimum schedule has not been determined 4 week schedule- administration intravenously at 1000 mg/m^2 over 30 minutes on 3 week schedule- Gemzar administered intravenously at 1250 mg/m^2	4 week schedule- Days 1,8 and 15 of each 28-day cycle. Cisplatin intravenously at 100 mg/m^2 on day 1 after the infusion of Gemzar. 3 week schedule- Days 1 and 8 of each 21 day cycle. Cisplatin at dosage of 100 mg/m^2 administered

Therapeutic Agent	Administration	Dose	Intervals
		over 30 minutes	intravenously after administration of Gemzar on day 1.
carboplatin (Paraplatin®)	Intravenous	Single agent therapy: 360 mg/m^2 I.V. on day 1 (infusion lasting 15 minutes or longer) Other dosage calculations: Combination therapy with cyclophosphamide, Dose adjustment recommendations, Formula dosing, etc.	Every 4 weeks
ifosamide (Ifex®)	Intravenous	1.2 g/m^2 daily	5 consecutive days Repeat every 3 weeks or after recovery from hematologic toxicity
topotecan hydrochloride (Hycamtin®)	Intravenous	1.5 mg/m^2 by intravenous infusion over 30 minutes daily	5 consecutive days, starting on day 1 of 21 day course

[00281] The invention also encompasses administration of the EphA2 vaccines of the invention in combination with radiation therapy comprising the use of x-rays, gamma rays and other sources of radiation to destroy the cancer cells. In preferred embodiments, the radiation treatment is administered as external beam radiation or teletherapy wherein the radiation is directed from a remote source. In other preferred embodiments, the radiation treatment is administered as internal therapy or brachytherapy wherein a radioactive source is placed inside the body close to cancer cells or a tumor mass.

[00282] Cancer therapies and their dosages, routes of administration and recommended usage are known in the art and have been described in such literature as the *Physician's Desk Reference* (56th ed., 2002).

5.10 Characterization And Demonstration Of Therapeutic Or Prophylactic Utility

[00283] Toxicity and efficacy of the prophylactic and/or therapeutic protocols of the instant invention can be determined by standard pharmaceutical procedures in experimental animals, *e.g.*, for determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD_{50}/ED_{50}. Prophylactic and/or therapeutic agents that exhibit large therapeutic indices are preferred. While prophylactic and/or therapeutic agents that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such agents to the site

of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

[00284] The data obtained from the animal studies can be used in formulating a range of dosage of the prophylactic and/or therapeutic agents for use in humans. The dosage of such agents lies preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any agent used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC_{50} (*i.e.*, the concentration of the vaccine or test compound that achieves a half-maximal inhibition of symptoms) as determined in animal studies. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

[00285] The anti-cancer activity of the therapies used in accordance with the present invention also can be determined by using various experimental animal models for the study of cancer, such as an immunocompetent mouse model, *e.g.*, Balb/c or C57/Bl/6, or transgenic mice where a mouse EphA2 is replaced with the human EphA2, mouse models to which murine tumor cell lines engineered to express human EphA2 are administered, animal models described in Section 6 *infra*, or any animal model (including hamsters, rabbits, etc.) known in the art and described in *Relevance of Tumor Models for Anticancer Drug Development* (1999, eds. Fiebig and Burger); *Contributions to Oncology* (1999, Karger); *The Nude Mouse in Oncology Research* (1991, eds. Boven and Winograd); and *Anticancer Drug Development Guide* (1997 ed. Teicher), herein incorporated by reference in their entireties.

[00286] Compounds for use in therapy can also be tested in other suitable animal model systems prior to testing in humans, including but not limited to in rats, mice, chicken, cows, monkeys, rabbits, hamsters, etc., for example, the animal models described above. The compounds can then be used in the appropriate clinical trials.

[00287] Further, any assays known to those skilled in the art can be used to evaluate the prophylactic and/or therapeutic utility of the vaccines and combinatorial therapies disclosed herein for treatment or prevention of hyperproliferative disorders such as cancer.

5.11 Vaccine Compositions

[00288] The compositions of the invention include bulk drug compositions useful in the manufacture of non-pharmaceutical compositions (*e.g.*, impure or non-sterile

compositions) and pharmaceutical compositions (*i.e.*, compositions that are suitable for administration to a subject or patient) which can be used in the preparation of unit dosage forms. Such compositions comprise a prophylactically or therapeutically effective amount of a prophylactic and/or therapeutic agent disclosed herein or a combination of those agents and a pharmaceutically acceptable carrier. Preferably, compositions of the invention comprise a prophylactically or therapeutically effective amount of one or more EphA2 vaccines of the invention. The EphA2 vaccines of the invention may comprise one or more EphA2 antigenic peptides of the invention and a pharmaceutically acceptable carrier, one or more EphA2 antigenic peptide expression vehicles of the invention and a pharmaceutically acceptable carrier, or one or more antigen presenting cells sensitized with an EphA2 antigenic peptide and a pharmaceutically acceptable carrier.

[00289] Where an EphA2 vaccine of the invention comprises an EphA2 antigenic peptides, the EphA2 antigenic peptide of the invention can be modified. For example, in certain embodiments, the EphA2 antigenic peptide may be formulated with lipid as a lipopeptide or linked to a carrier molecule (and/or polymerized).

[00290] In a further embodiment, the composition of the invention further comprises an additional prophylactic or therapeutic, *e.g.*, anti-cancer, agent.

[00291] In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant (*e.g.*, Freund's adjuvant (complete and incomplete) or, more preferably, MF59C.1 adjuvant available from Chiron, Emeryville, CA), excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like.

[00292] Generally, the ingredients of compositions of the invention are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

[00293] The compositions of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

[00294] Various delivery systems are known and can be used to administer an EphA2 vaccine of the invention or the combination of an EphA2 vaccine of the invention and a prophylactic agent or therapeutic agent useful for preventing or treating cancer, *e.g.*, encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the EphA2 antigenic peptide, receptor-mediated endocytosis (see, *e.g.*, Wu and Wu, 1987, *J. Biol. Chem.* 262:4429-4432), construction of a nucleic acid as part of a retroviral or other vector, etc. Methods of administering an EphA2 vaccine or the combination of an EphA2 vaccine of the invention and prophylactic or therapeutic agent, but are not limited to, parenteral administration (*e.g.*, intradermal, intramuscular, intraperitoneal, intravenous and subcutaneous), epidural, and mucosal (*e.g.*, intranasal, inhaled, and oral routes). In a specific embodiment, an EphA2 vaccine of the invention or the combination of an EphA2 vaccine of the invention and prophylactic or therapeutic agent are administered intramuscularly, intravenously, or subcutaneously. The EphA2 vaccine of the invention or the combination of an EphA2 vaccine of the invention and prophylactic or therapeutic agent may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.*, oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local.

[00295] In a specific embodiment, it may be desirable to administer the EphA2 vaccine of the invention or the combination of an EphA2 vaccine of the invention and prophylactic or therapeutic agents of the invention locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion, by

injection, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers.

[00296] In yet another embodiment, the EphA2 vaccine of the invention or the combination of an EphA2 vaccine of the invention and prophylactic or therapeutic agent can be delivered in a controlled release or sustained release system. In one embodiment, a pump may be used to achieve controlled or sustained release (see Langer, *supra*; Sefton, 1987, *CRC Crit. Ref. Biomed. Eng.* 14:20; Buchwald et al., 1980, *Surgery* 88:507; Saudek et al., 1989, *N. Engl. J. Med.* 321:574). In another embodiment, polymeric materials can be used to achieve controlled or sustained release of the EphA2 antigenic peptides of the invention (see *e.g.*, Medical Applications of Controlled Release, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida (1974); Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, 1983, *J. Macromol. Sci. Rev. Macromol. Chem.* 23:61; see also Levy et al., 1985, *Science* 228:190; During et al., 1989, *Ann. Neurol.* 25:351; Howard et al., 1989, *J. Neurosurg.* 7 1:105); U.S. Patent Nos. 5,679,377; 5,916,597; 5,912,015; 5,989,463; 5,128,326; International Publication Nos. WO 99/15154 and WO 99/20253. Examples of polymers used in sustained release formulations include, but are not limited to, poly(2-hydroxy ethyl methacrylate), poly(methyl methacrylate), poly(acrylic acid), poly(ethylene-co-vinyl acetate), poly(methacrylic acid), polyglycolides (PLG), polyanhydrides, poly(N-vinyl pyrrolidone), poly(vinyl alcohol), polyacrylamide, poly(ethylene glycol), polylactides (PLA), poly(lactide-co-glycolides) (PLGA), and polyorthoesters. In a preferred embodiment, the polymer used in a sustained release formulation is inert, free of leachable impurities, stable on storage, sterile, and biodegradable. In yet another embodiment, a controlled or sustained release system can be placed in proximity of the prophylactic or therapeutic target, thus requiring only a fraction of the systemic dose (see, *e.g.*, Goodson, in Medical Applications of Controlled Release, supra, vol. 2, pp. 115-138 (1984)).

[00297] Controlled release systems are discussed in the review by Langer (1990, Science 249:1527-1533). Any technique known to one of skill in the art can be used to produce sustained release formulations comprising one or more therapeutic agents of the invention. See, *e.g.*, U.S. Patent No. 4,526,938; International Publication Nos. WO 91/05548 and WO 96/20698; Ning et al., 1996, *Radiotherapy & Oncology* 39:179-189; Song et al., 1995, *PDA Journal of Pharmaceutical Science & Technology* 50:372-397; Cleek et al., 1997, *Pro. Int'l. Symp. Control. Rel. Bioact. Mater.* 24:853-854; and Lam et al., 1997, *Proc. Int'l. Symp. Control Rel. Bioact. Mater.* 24:759-760, each of which is incorporated herein by reference in its entirety.

5.11.1 Formulations

[00298] Pharmaceutical compositions for use in accordance with the present invention may be formulated in conventional manner using one or more physiologically acceptable carriers or excipients.

[00299] Thus, the EphA2 antigenic peptides of the invention and their physiologically acceptable salts and solvates (or EphA2 antigenic peptide expression vehicles or antigen presenting cells sensitized with an EphA2 antigenic peptide) may be formulated for administration by inhalation or insufflation (either through the mouth or the nose) or oral, parenteral or mucosal (such as buccal, vaginal, rectal, sublingual) administration. In a preferred embodiment, local or systemic parenteral administration is used.

[00300] For oral administration, the vaccine may take the form of, for example, tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (*e.g.*, pregelatinised maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (*e.g.*, lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (*e.g.*, magnesium stearate, talc or silica); disintegrants (*e.g.*, potato starch or sodium starch glycolate); or wetting agents (*e.g.*, sodium lauryl sulphate). The tablets may be coated by methods well known in the art. Liquid preparations for oral administration may take the form of, for example, solutions, syrups or suspensions, or they may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (*e.g.*, sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (*e.g.*, lecithin or acacia); non-aqueous vehicles (*e.g.*, almond oil, oily esters, ethyl alcohol or fractionated vegetable oils); and preservatives (*e.g.*, methyl or propyl-p-hydroxybenzoates or sorbic acid). The preparations may also contain buffer salts, flavoring, coloring and sweetening agents as appropriate.

[00301] Preparations for oral administration may be suitably formulated to give controlled release of the active compound.

[00302] For buccal administration the compositions may take the form of tablets or lozenges formulated in conventional manner.

[00303] For administration by inhalation, the prophylactic or therapeutic agents for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, *e.g.*, dichlorodifluoromethane, trichlorofluoromethane,

dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of *e.g.*, gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

[00304] The EphA2 vaccine may be formulated for parenteral administration by injection, *e.g.*, by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, *e.g.*, in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, *e.g.*, sterile pyrogen-free water, before use.

[00305] The vaccines of the invention may also be formulated in rectal compositions such as suppositories or retention enemas, *e.g.*, containing conventional suppository bases such as cocoa butter or other glycerides.

[00306] In addition to the formulations described previously, the prophylactic or therapeutic agents may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the prophylactic or therapeutic agents may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

[00307] The invention also provides that an EphA2 vaccine of the invention is packaged in a hermetically sealed container such as an ampoule or sachette indicating the quantity. In one embodiment, the vaccine is supplied as a dry sterilized lyophilized powder or water free concentrate in a hermetically sealed container and can be reconstituted, *e.g.*, with water or saline to the appropriate concentration for administration to a subject.

[00308] In a preferred embodiment of the invention, the formulation and administration of various chemotherapeutic, biological/immunotherapeutic and hormonal therapeutic agents for use in combination with the vaccine of the invention are known in the art and often described in the *Physician's Desk Reference, 56th ed.* (2002). For instance, in certain specific embodiments of the invention, the agents can be formulated and supplied as provided in Table 2.

[00309] In other embodiments of the invention, radiation therapy agents such as radioactive isotopes can be given orally as liquids in capsules or as a drink. Radioactive

isotopes can also be formulated for intravenous injections. The skilled oncologist can determine the preferred formulation and route of administration.

[00310] In certain embodiments the EphA2 antigenic peptides and anti-idiotypic antibodies of the invention are formulated at 1 mg/ml, 5 mg/ml, 10 mg/ml, and 25 mg/ml for intravenous injections and at 5 mg/ml, 10 mg/ml, and 80 mg/ml for repeated subcutaneous administration and intramuscular injection.

[00311] The compositions may, if desired, be presented in a pack or dispenser device that may contain one or more unit dosage forms containing the active ingredient. The pack may for example comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration.

5.11.2 Dosages

[00312] The amount of the composition of the invention which will be effective in the treatment, prevention or management of cancer can be determined by standard research techniques. For example, the dosage of the EphA2 vaccine of the invention which will be effective in the treatment, prevention or management of cancer can be determined by administering the composition to an animal model such as, *e.g.*, the animal models disclosed herein or known to those skilled in the art. In addition, *in vitro* assays may optionally be employed to help identify optimal dosage ranges.

[00313] Selection of the preferred effective dose can be determined (*e.g.*, via clinical trials) by a skilled artisan based upon the consideration of several factors which will be known to one of ordinary skill in the art. Such factors include the disease to be treated or prevented, the symptoms involved, the patient's body mass, the patient's immune status and other factors known by the skilled artisan to reflect the accuracy of administered pharmaceutical compositions.

[00314] The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the cancer, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model test systems.

[00315] For EphA2 antigenic peptides or anti-idiotypic antibodies, the dosage administered to a patient is typically 0.1 mg/kg to 100 mg/kg of the patient's body weight. Preferably, the dosage administered to a patient is between 0.1 mg/kg and 20 mg/kg of the patient's body weight, more preferably 1 mg/kg to 10 mg/kg of the patient's body weight.

[00316] With respect to the dosage of bacterial EphA2 vaccines of the invention, the dosage is based on the amount colony forming units (c.f.u.). Generally, in various

embodiments, the dosage ranges are from about 1.0 c.f.u./kg to about 1 x 10^{10} c.f.u./kg; from about 1.0 c.f.u./kg to about 1 x 10^{8} c.f.u./kg; from about 1 x 10^{2} c.f.u./kg to about 1 x 10^{8} c.f.u./kg; and from about 1 x 10^{4} c.f.u./kg to about 1 x 10^{8} c.f.u./kg. Effective doses may be extrapolated from dose-response curves derived animal model test systems. In certain exemplary embodiments, the dosage ranges are 0.001-fold to 10,000-fold of the murine LD_{50}, 0.01-fold to 1,000-fold of the murine LD_{50}, 0.1-fold to 500-fold of the murine LD_{50}, 0.5-fold to 250-fold of the murine LD_{50}, 1-fold to 100-fold of the murine LD_{50}, and 5-fold to 50-fold of the murine LD_{50}. In certain specific embodiments, the dosage ranges are 0.00.1-fold, 0.01-fold, 0.1-fold, 0.5-fold, 1-fold, 5-fold, 10-fold, 50-fold, 100-fold, 200-fold, 500-fold, 1,000-fold, 5,000-fold or 10,000-fold of the murine LD_{50}.

[00317] For other cancer therapeutic agents administered to a patient, the typical doses of various cancer therapeutics known in the art are provided in Table 2. Given the invention, certain preferred embodiments will encompass the administration of lower dosages in combination treatment regimens than dosages recommended for the administration of single agents.

[00318] The invention provides for any method of administrating lower doses of known prophylactic or therapeutic agents than previously thought to be effective for the prevention, treatment, management or amelioration of cancer. Preferably, lower doses of known anti-cancer therapies are administered in combination with lower doses of EphA2 vaccines of the invention.

5.12 Kits

[00319] The invention provides a pack or kit comprising one or more containers filled with an EphA2 vaccine of the invention or a component of an EphA2 vaccine of the invention. Additionally, one or more other prophylactic or therapeutic agents useful for the treatment of a cancer or other hyperproliferative disorder can also be included in the pack or kit. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

[00320] The present invention provides kits that can be used in the above methods. In one embodiment, a kit comprises one or more a EphA2 vaccines of the invention. In another embodiment, a kit further comprises one or more other prophylactic or therapeutic agents useful for the treatment of cancer or another hyperproliferative disorder, in one or more containers. In other embodiments, the prophylactic or therapeutic agent is a biological or hormonal therapeutic.

6. EXAMPLES: *LISTERIA*-BASED EPHA2 VACCINES PROVIDE THERAPEUTIC AND PROPHYLACTIC BENEFITS AGAINST EPHA2-EXPRESSING CANCERS

[00321] Given the mechanisms by which *Listeria* programs the presentation of heterologous antigens via the MHC class I pathway, the efficiency of both expression of heterologous genes and secretion of the newly synthesized protein from the bacterium into the cytoplasm of the infected (antigen presenting) cell is related directly to the potency of CD8+ T cell priming and/or activation. As the level of Ag-specific T cell priming is related directly to vaccine efficacy, the efficiency of heterologous protein expression and secretion is linked directly to vaccine potency. Thus, the efficiency of EphA2 expression and secretion was optimized to maximize the potency of *Listeria*-based vaccines, in terms of priming and/or activating CD8+ T cell responses specific for the encoded EphA2 protein.

[00322] A mouse immunotherapy model was created for testing the *Listeria*-based vaccines of the invention. Two murine tumor cell lines, the CT26 murine colon carcinoma cell line, and the B16F10 murine melanoma cell line, were created to express high levels of the huEphA2 protein. FACS cell sorting assays were performed to identify CT26 and B16F10 tumor cells expressing high levels of huEphA2, which were pooled and analyzed by Western blot analysis. Clones were further pooled by FACS cell sorting to generate subclones expressing the highest levels of huEphA2.

[00323] Immunoassays were also performed, including intracellular cytokine staining (ICS) assays to measure Th1-cytokine production, and ELISPOT assays to measure Th1-cytokine specific induction in murine splenocytes following *Listeria* vaccination.

6.1 EXAMPLE 1: CONSTRUCTION OF EphA2-EXPRESSING AND CONTROL *LISTERIA* STRAINS

6.1.1 Preparation of mutant *Listeria* strains.

[00324] *Listeria* strains were derived from 10403S (Bishop et al., *J. Immunol.* 139:2005 (1987)). *Listeria* strains with in-frame deletions of the indicated genes were generated by SOE-PCR and allelic exchange with established methods (Camilli, et al, *Mol. Microbiol.* 8:143 (1993)). The mutant strain LLO L461T (DP-L4017) was described in Glomski, et al, *J. Cell. Biol.* 156: 1029 (2002), incorporated by reference herein. The *actA*$^-$ mutant (DP-L4029) is the DP-L3078 strain described in Skoble et al., *J. of Cell Biology*, 150: 527-537 (2000), incorporated by reference herein in its entirety, which has been cured of its prophage. (Prophage curing is described in (Lauer *et al.*, *J. Bacteriol.* 2002, 184:4177 (2002)).

[00325] In some vaccines, mutant strains of Listeria that are deficient with respect to internalin B (Genbank accession number AL591975 (*Listeria monocytogenes* strain EGD, complete genome, segment 3/12; inlB gene region: nts. 97008-98963), incorporated by reference herein in its entirety) are used. One particular *actA⁻inlB⁻* strain (DP-L4029*inlB*) was deposited with the American Type Culture Collection (ATCC) on October 3, 2003, and designated with accession number PTA-5562).

6.1.2 Cloning vectors

[00326] Selected heterologous antigen expression cassette molecular constructs were inserted into pPL2 (Lauer *et al.*, 2002, J. Bacteriol.), or pAM401 (Wirth *et. al.*, *J. Bacteriol.* 165:831-836), modified to contain the multiple cloning sequence of pPL2 (Aat II small fragment, 171 bps), inserted between blunted *Xba I* and *Nru I* recognition sites, within the tetracycline resistance gene (pAM401-MCS). In general, the hly promoter and (selected) signal peptide sequence was inserted between the unique *Kpn I* and *Bam HI* sites in the pPL2 or pAM401-MCS plasmid vectors. Selected EphA2 genes (sometimes modified to contain N-terminal and C-terminal epitope tags; *see* description below) were cloned subsequently into these constructs between unique *Bam HI* and *Sac I* sites. Molecular constructs based on the pAM401-MCS plasmid vector were introduced by electroporation into selected *Listeria* monocytogenes strains also treated with lysozyme, utilizing methods common to those skilled in the art. The expected plasmid structure in *Listeria*-transfectants was verified by isolating DNA from colonies that formed on chloramphenicol-containing BHI agar plates (10 μg/ml) by restriction enzyme analysis. Recombinant *Listeria* transformed with various pAM401-MCS based heterologous protein expression cassette constructs were utilized to measure heterologous protein expression and secretion, as described below.

[00327] The pPL2 based heterologous protein expression cassette constructs were incorporated into the tRNAArg gene in the genome of selected *Listeria* strains, according to the methods as described previously (Lauer *et al.*, 2002, *J. Bacteriol.* 184:4177-4186). Briefly, the pPL2 heterologous protein expression cassette constructs plasmid was first introduced into the *E. coli* host strain SM10 (Simon *et al.*,1983, *Bio/Technology* 1:784-791) by electroporation or by chemical means. Subsequently, the pPL2-based plasmid was transferred from transformed SM10 to the selected *Listeria* strains by conjugation. Following incubation on drug-selective BHI agar plates containing 7.5 μg of chloramphenicol per ml and 200 μg of streptomycin per ml as described, selected colonies are purified by passaging 3 times on plates with the same composition. To verify integration of the pPL2 vector at the phage attachment site, individual colonies are picked

and screened by PCR using the primer pair of forward primer NC16 (5'-gtcaaaacatacgctcttatc-3') (SEQ ID NO:47) and reverse primer PL95 (5'-acataatcagtccaaagtagatgc-3') (SEQ ID NO:48). Selected colonies having the pPL2-based plasmid incorporated into the tRNAArg gene in the genome of selected *Listeria* strains yielded a diagnostic DNA amplicon of 499 bps.

6.1.3 Promoter

[00328] Heterologous protein expression cassettes contained the prfA-dependent hly promoter, which drives the transcription of the gene encoding Listeriolysin O (LLO), and is activated within the microenvironment of the infected cell. Nucleotides 205586-206000 (414 bps) were amplified by PCR from *Listeria* monocytogenes, strain DP-L4056, using the primer pair shown below. The region amplified includes the hly promoter and also the first 28 amino acids of LLO, comprising the secA1 signal peptide (*ibid*) and PEST domain. The expected sequence of this region for *Listeria monocytogenes*, strain EGD can be found in GenBank (Accession number: gi|16802048|ref|NC_003210.1|[16802048]).

[00329] Primer Pair

Forward (KpnI-LLO nts. 1257-1276):

5'-CTCTGGTACCTCCTTTGATTAGTATATTC (SEQ ID NO:49)

Reverse (Bam HI-LLO nts. X-x):

5'-CTCTGGATCCATCCGCGTGTTTCTTTTCG (SEQ ID NO:50)

(Restriction endonuclease recognition sites are underlined)

[00330] The 422 bp PCR amplicon was cloned into the plasmid vector pCR-XL-TOPO (Invitrogen, Carlsbad, CA), according to the manufacturer's specifications. The nucleotide sequences of *Listeria*-specific bases in the pCR-XL-TOPO-hly promoter plasmid clone was determined. *Listeria* monocytogenes strain DP-L4056 contained eight nucleotide base changes flanking the prfA box in the hly promoter, as compared to the EGD strain. The *hly* promoter alignment for the *Listeria* monocytogenes DP-L4056 and EGD strains is shown in the Figure below (SEQ ID NOs:68 and 69, respectively).

Listeria hly DP-L4056 and EGD Alignment

```
Query:          Listeria EGD
Subject:        DP-L4056 (wild-type, Portnoy strain)

                                                                    prfA Box
Query: 1    ggtacctccttttgattagtatattcctatcttaaagtgacttttatgttgaggcattaac 60
            ||||||||||||||||||||||||||||||||||||||| |||||||||| ||||||||||
Sbjct: 1    ggtacctccttttgattagtatattcctatcttaaagttacttttatgtggaggcattaac 60


Query: 61   atttgttaacgacgataaagggacagcaggactagaataaagctataaagcaagcatata 120
            ||||||||| ||||  ||| ||| |||| ||||||||||||||||||||||||||||||
Sbjct: 61   atttgttaatgacgtcaaaaggatagcaagactagaataaagctataaagcaagcatata 120


Query: 121  atattgcgtttcatctttagaagcgaatttcgccaatattataattatcaaaagagaggg 180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 121  atattgcgtttcatctttagaagcgaatttcgccaatattataattatcaaaagagaggg 180

                                                  Shine-Delgarno     LLO start
Query: 181  gtggcaaacggtatttggcattattaggttaaaaaatgtagaaggagagtgaaacccatg 240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 181  gtggcaaacggtatttggcattattaggttaaaaaatgtagaaggagagtgaaacccatg 240
```

[00331] The 422 bp DNA corresponding to the hly promoter and secA1 LLO signal peptide were liberated from the pCR-XL-TOPO-hly promoter plasmid clone by digestion with Kpn I and Bam HI, and cloned into the pPL2 plasmid vector (Lauer *et al.*, 2002, *J. Bact.*), according to conventional methods well-known to those skilled in the art. This plasmid is known as pPL2-hlyP (native).

6.1.4 Cloning and Insertion of EphA2 into pPL2 vectors for expression in selected recombinant *Listeria monocytogenes* strains

[00332] The external (EX2) and cytoplasmic (CO) domains of EphA2 which flank the EphA2 transmembrane helix were cloned separately for insertion into various pPL2-signal peptide expression constructs. Genes corresponding to the native mammalian sequence or codon-optimized for expression in *Listeria monocytogenes* of EphA2 EX2 and CO domains were used. The optimal codons in *Listeria* (see table, *ibid*) for each of the 20 amino acids were utilized for codon-optimized EphA2 EX2 and EphA2 CO. The codon-optimized EphA2 EX2 and CO domains were synthesized by extension of overlapping oligonucleotides, using techniques common to those skilled in the art. The expected sequence of all synthesized EphA2 constructs was verified by nucleotide sequencing.

[00333] SEQ ID NOS:23, 21 and 22 represent the primary amino acid sequences, together with the native and codon-optimized nucleotide sequences, respectively, for the EX2 domain of EphA2.

[00334] SEQ ID NOS: 34, 32 and 33 represent the primary amino acid sequences, together with the native and codon-optimized nucleotide sequences, respectivley, for the CO domain of EphA2.

[00335] Additonally, FLAG (Stratagene, La Jolla, CA) and myc epitope tags were inserted, respectively, in-frame at the amino and carboxy termini of synthesized EphA2

EX2 and CO genes for detection of expressed and secreted EphA2 by Western blot analysis using antibodies specific for the FLAG or proteins. Thus, the expressed protein had the following ordered elements: NH_2-Signal Peptide-FLAG-EphA2-myc-CO_2. Shown below are the FLAG and myc epitope tag amino acid and codon-optimized nucleotide sequences.

[00336] FLAG

5'-GATTATAAAGATGATGATGATAAA (SEQ ID NO:51)

NH_2-DYKDDDDK-CO_2 (SEQ ID NO:52)

[00337] Myc

5'-GAACAAAAATTAATTAGTGAAGAAGATTTA (SEQ ID NO:53)

NH_2-EQKLISEEDL-CO_2 (SEQ ID NO:54)

6.1.5 Detection of synthesized and secreted heterologous proteins by Western blot analysis

[00338] Synthesis of EphA2 protein and secretion from various selected recombinant *Listeria*-EphA2 strains was determined by Western blot analysis of trichloroacetic acid (TCA) precipitated bacterial culture fluids. Briefly, mid-log phase cultures of *Listeria* grown in BHI media were collected in a 50 mL conical centrifuge tube, the bacteria were pelleted, and ice-cold TCA was added to a final [6%] concentration to the bacterial culture supernatant and incubated on ice minimally for 90 min or overnight. The TCA-precipitated proteins were collected by centrifugation at 2400 X g for 20 min at 4°C. The pellet was then resuspended in 300-600 μl volume of TE, pH 8.0 containing 15 μg/ml phenol red. Sample dissolution was facilitated by vortexing. Sample pH was adjusted by NH_4OH addition if necessary until color was pink. All samples were prepared for electrophoresis by addition of 100 μl of 4X SDS loading buffer and incubating for 10 min. at 90°C. The samples were then centrifuged from 5 min at 14,000 rpm in a micro-centrifuge, and the supernatants collected and stored at –20°C. For Western bolt analysis, 20 μl of prepared fractions (the equivalent of culture fluids from of 1-4 x 10^9 bacteria), were loaded on the 4-12% SDS-PAGE gel, electrophoresed, and the proteins were transferred to PDDF membrane, according to common methods used by those skilled in the art. Transferred membranes were prepared s for incubation with antibody, by incubating in 5% dry milk in PBS for 2 hr. at room temperature with agitation. Antibodies were used under the following dilutions in PBST buffer (0.1% Tween 20 in PBS): (1) Rabbit anti-Myc polyclonal antibody (ICL laboratories, Newberg, Oregon) at 1:10,000; (2) murine anti-FLAG monoclonal antibody (Stratagene, *ibid*) at 1:2,000; and, (3) Rabbit anti-EphA2 (carboxy terminus-specific) polyclonal antibody (sc-924, Santa Cruz Biotechnology, Inc., Santa Cruz, CA). Specific binding of antibody to protein targets was evaluated by

secondary incubation with goat anti-rabbit or anti-mouse antibody conjugated with horseradish peroxidase and detection with the ECL chemilumenescence assay kit (Amersham), and exposure to film.

6.1.6 Secretion of EphA2 protein by recombinant *Listeria* encoding various forms of EphA2

6.1.6.1. *Listeria*: [strains DP-L4029 (*actA*) or DP-L4017 (LLO L461T)]

[00339] Expression cassette construct: LLOss-PEST-CO-EphA2 (SEQ ID NO:35)

[00340] The native sequence of the EphA2 CO domain was genetically fused to the native secA1 LLO sequence, and the heterologous antigen expression cassette under control of the *Listeria hly* promoter was inserted into the pPL2 plasmid between the *Kpn I* and *Sac I* sites as described (*ibid*). The pPL2-EphA2 plasmid constructs were introduced by conjugation into the *Listeria* strains DP-L4029 (actA) and DP-L4017 (L461T LLO) as described (*ibid*). Figure 1 shows the results of a Western blot analysis of TCA-precipitated bacterial culture fluids of 4029-EphA2 CO and 4017-EphA2 CO. This analysis demonstrated that recombinant *Listeria* engineered to contain a heterologous protein expression cassette comprised of native sequences corresponding to the secA1 and EphA2 CO fusion protein secreted multiple EphA2-specific fragments that were lower than the 52 kDa expected molecular weight, demonstrating the need for modification of the expression cassette.

6.1.6.2. *Listeria*: [DP-L4029 (*actA*)]

[00341] Expression cassette constructs:

Native LLOss-PEST-FLAG-EX2_EphA2-myc-CodonOp (SEQ ID NO:26)

(CodonOp) LLOss-PEST-(CodonOp)FLAG-EX2_EphA2-myc (SEQ ID NO:28)

[00342] The native secA1 LLO signal peptide sequence or secA1 LLO signal peptide sequence codon-optimized for expression in *Listeria* was fused genetically with the EphA2 EX2 domain sequence codon-optimized for expression in *Listeria*, and the heterologous antigen expression cassette under control of the *Listeria hly* promoter was inserted into the pPL2 plasmid between the *Kpn I* and *Sac I* sites as described (*ibid*). The pPL2-EphA2 plasmid constructs were introduced by conjugation into the *Listeria* strain DP-L4029 (actA) as described (*ibid*). Figure 2 shows the results of a Western blot analysis of TCA-precipitated bacterial culture fluids of *Listeria* actA encoding either the native or codon-optimized secA1 LLO signal peptide fused with the codon-optimized EphA2 EX2 domain.

This analysis demonstrated that the combination of utilizing sequence for both signal peptide and heterologous protein optimized for the preferred codon usage in *Listeria monocytogenes* resulted in expression of the expected full-length EphA2 EX2 domain protein. Expression of full-length EphA2 EX2 domain protein was poor with codon-optimization of the EphA2 coding sequence alone. The level of heterologous protein expression (fragmented or full-length) was highest when utilizing the *Listeria monocytogenes* LLO secA1 signal peptide, codon-optimized for expression in *Listeria monocytogenes*.

6.1.6.3. *Listeria*: [DP-L4029 (*actA*)]

[00343] Expression cassette constructs:

Native LLOss-PEST-(CodonOp) FLAG-EphA2_CO-myc (SEQ ID NO:37)

CodonOp LLOss-PEST-(CodonOp) FLAG- EphA2_CO-myc (SEQ ID NO:39)

CodonOp PhoD-(CodonOp) FLAG- EphA2_CO-myc (SEQ ID NO:41)

[00344] The native secA1 LLO signal peptide sequence or the secA1 LLO signal peptide sequence codon-optimized for expression in *Listeria*, or, alternatively, the Tat signal peptide of the phoD gene from *Bacillus subtilis* codon-optimized for expression in *Listeria*, was fused genetically with the EphA2 CO domain sequence codon-optimized for expression in *Listeria,* and the heterologous antigen expression cassette under control of the *Listeria hly* promoter was inserted into the pAM401-MCS plasmid between the *Kpn I* and *Sac I* sites as described (*ibid*). The pAM401-EphA2 plasmid constructs were introduced by electroporation into the *Listeria* strain DP-L4029 (actA) as described (*ibid*). Figure 3 shows the results of a Western blot analysis of TCA-precipitated bacterial culture fluids of *Listeria* actA encoding either the native or codon-optimized secA1 LLO signal peptide, or codon-optimized *Bacillus subtilis phoD* Tat signal peptide fused with the codon-optimized EphA2 CO domain. This analysis demonstrated once again that the combination of utilizing sequence for both signal peptide and heterologous protein optimized for the preferred codon usage in *Listeria monocytogenes* resulted in expression of the expected full-length EphA2 CO domain protein. Furthermore, expression and secretion of the expected full-length EphA2 CO domain protein resulted from recombinant *Listeria* encoding codon-optimized *Bacillus subtilis phoD* Tat signal peptide fused with the codon-optimized EphA2 CO domain. This result demonstrates the novel and unexpected finding that signal peptides from distinct bacterial species can be utilized to program the secretion of heterologous proteins from recombinant *Listeria*. Expression of full-length EphA2 CO domain protein

was poor with codon-optimization of just the EphA2 sequence. The level of heterologous protein expression was highest when utilizing signal peptides codon-optimized for expression in *Listeria monocytogenes*.

6.1.7 Construction of *Listeria* strains expressing AH1/OVA or AH1-A5/OVA

[00345] Mutant *Listeria* strains expressing a truncated form of a model antigen ovalbumin (OVA), the immunodominant epitope from mouse colorectal cancer (CT26) known as AH1 (SPSYVYHQF) (SEQ ID NO:55), and the altered epitope AH1-A5 (SPSYAYHQF (SEQ ID NO:56); Slansky et al., *Immunity*, 13:529-538 (2000)) were prepared. The pPL2 integrational vector (Lauer et al., *J. Bacteriol.* 184:4177 (2002); U.S. Patent Publication No. 2003/0203472) was used to derive OVA and AH1-A5/OVA recombinant *Listeria* strains containing a single copy integrated into an innocuous site of the *Listeria* genome.

[00346] Construction of OVA-expressing Listeria (DP-L4056)An antigen expression cassette consisting of hemolysin-deleted LLO fused with truncated OVA and contained in the pPL2 integration vector (pPL2/LLO-OVA) is first prepared. The *Listeria*-OVA vaccine strain is derived by introducing pPL2/LLO-OVA into the phage-cured *L. monocytogenes* strain DP-L4056 at the PSA (**P**hage from Scott**A**) attachment site $tRNA^{Arg}$-*attBB'*.

[00347] PCR is used to amplify the hemolysin-deleted LLO using the following template and primers:

Source: DP-L4056 genomic DNA

Primers:

Forward (*KpnI*-LLO nts. 1257-1276):

5'-CTCTGGTACCTCCTTTGATTAGTATATTC (SEQ ID NO:57)

(T_m:LLO-spec: 52°C. Overall: 80°C.)

Reverse (*BamHI-XhoI*-LLO nts. 2811-2792):

5'-CAATGGATCCCTCGAGATCATAATTTACTTCATCCC

(SEQ ID NO:58)

(T_m:LLO-spec: 52°C. Overall: 102°C.)

[00348] PCR is also used to amplify the truncated OVA using the following template and primers:

Source: pDP3616 plasmid DNA from DP-E3616 *E. coli* (Higgins et al., *Mol. Molbiol.* 31:1631-1641 (1999)).

Primers:

Forward (*XhoI- NcoI* OVA cDNA nts. 174-186):

5'-ATTTCTCGAGTCCATGGGGGGTTCTCATCATC

(SEQ ID NO:59)

(T_m: OVA-spec: 60°C. Overall: 88°C.)

Reverse (*XhoI-NotI-HindIII*):

5'-GGTGCTCGAGTGCGGCCGCAAGCTT (SEQ ID NO:60)

(T_m: Overall: 82°C.)

[00349] One protocol for completing the construction process involves first cutting the LLO amplicon with KpnI and BamHI and inserting the KpnI/BamHI vector into the pPL2 vector (pPL2-LLO). The OVA amplicon is then cut with *XhoI* and *NotI* and inserted into the pPL2-LLO which has been cut with *XhoI/NotI*. (Note: The pPL2 vector does not contain any *XhoI* sites; pDP-3616 contains one *XhoI* site, that is exploited in the OVA reverse primer design.) The construct pPL2/LLO-OVA is verified by restriction analysis (*KpnI*-LLO-*XhoI*-OVA-*NotI*) and sequencing. The plasmid pPL2/LLO-OVA is introduced into *E. coli* by transformation, followed by introduction and integration into *Listeria* (DP-L4056) by conjugation, exactly as described by Lauer *et al.* (or into another desired strain of *Listeria*).

[00350] Construction of Listeria strains expressing AH1/OVA or AH1-A5/OVATo prepare *Listeria* expressing either the AH1/OVA or the AH1-A5/OVA antigen sequences, inserts bearing the antigen are first prepared from oligonucleotides and then ligated into the vector pPL2-LLO-OVA (prepared as described above).

[00351] The following oligonucleotides are used in preparation of the AH1 or AH1-A5 insert:

AH1 epitope insert (ClaI-PstI compatible ends):

Top strand oligo (AH1 Top):

5'-CGATTCCCCTAGTTATGTTTACCACCAATTTGCTGCA

(SEQ ID NO:61)

Bottom strand oligo (AH1 Bottom):

5'-GCAAATTGGTGGTAAACATAACTAGGGGAAT (SEQ ID NO:62)

AH1-A5 epitope insert (ClaI-AvaII compatible ends):

[00352] The sequence of the AH1-A5 epitope is SPSYAYHQF (SEQ ID NO:56) (5'-AGT CCA AGT Tat GCA Tat CAT CAA TTT-3') (SEQ ID NO:63).

Top: 5'-**CGAT**AGTCCAAGTTATGCATATCATCAATTT**GC**

(SEQ ID NO:64)

Bottom: 5'-**GTCGC**AAATTGATGATATGCATAACTTGGACT**AT**

(SEQ ID NO:65)

[00353] The oligonucletide pair for a given epitope are mixed together at an equimolar ratio, heated at 95 °C for 5 min. The oligonucleotide mixture is then allowed to slowly cool. The annealed oligonucleotide pairs are then ligated at a 200 to 1 molar ratio with pPL2-LLO/OVA plasmid prepared by digestion with the relevant restriction enzymes. The identity of the new construct can be verified by restriction analysis and/or sequencing.

[00354] The plasmid can then be introduced into E. coli by transformation, followed by introduction and integration into *Listeria* (DP-L4056) by conjugation, exactly as described by Lauer et al., or into another desired strain of *Listeria*, such as an *actA*⁻ mutant strain (DP-L0429), LLO L461T strain (DP-L4017), or *actA*⁻/*inlB*⁻ strain (DP-L4029*inlB*).

6.2 EXAMPLE 2: GENERATION OF MURINE TUMOR CELL LINES THAT EXPRESS HUMAN EphA2

6.2.1 Selection of CT26 Murine Colon Carcinoma Cells Expressing High Levels of huEphA2

6.2.1.1. Transfection Assays With Lipofectamine™

[00355] CT26 cells were transfected with constructs containing huEphA2 using standard transfection techniques and commercially available Lipofectamine™ according to the manufacturer's instructions.

6.2.1.2. Flow Cytometry (FACS) Analysis

[00356] Single cell FACS sorting assays were performed by standard techniques to identify CT26 murine carcinoma tumor cell expressing high levels of human EphA2.

[00357] Figure 4 illustrates a representative experiment, showing that the EphA2-3 clone expressed the highest levels of human EphA2 protein.

6.2.1.3. Western Blot of Pooled Populations Expressing High Levels of huEphA2

[00358] Western blotting was also performed using standard techniques to determine the levels of human EphA2 protein expression in CT26 cells following FACS sorting of pooled populations of cells transfected with various constructs containing the huEphA2 gene. Figure 5 depicts results of a representative experiment. Compared to various clones tested, the huEphA2-3 clone expressed the highest levels of human EphA2 protein and was selected for the *in vivo* efficacy studies described below. Cells were further pooled to generate subclones expressing the highest levels of huEphA2.

6.2.2 Selection of B16F10 Murine Melanoma Cells Expressing High Levels of huEphA2

6.2.2.1. Retroviral Transduction

[00359] Human EphA2 was introduced into B16F10 murine melanoma cells by a retroviral transduction method to create clones expressing high levels of the protein.

6.2.2.2. Flow Cytometry (FACS) Analysis

[00360] As was performed on the CT26 cells, single cell FACS sorting assays were performed by standard techniques on B16F10 cells expressing huEphA2 to generate clones expressing high levels of huEphA2. Clones expressing the highest levels of huEphA2 were pooled and further examined by Western blot analysis. A representative FACS experiment is depicted in Figure 6, showing a B16F10 subclone expressing high levels of huEphA2.

6.2.3 Western Blot of Pooled Populations Expressing High Levels of huEphA2

[00361] Western blotting was also performed as described above to determine levels of huEphA2 protein expression in B16F10 cells following FACS sorting of pooled populations of cells containing the huEphA2 gene introduced by retroviral transduction. Cells were further pooled to generate subclones expressing the highest levels of huEphA2.

6.2.4 Transfection of 293 Cells with pCDNA4 plasmids encoding full-length EphA2

[00362] Expression cassette constructs:

[00363] pCDNA4-EphA2

[00364] The native full-length EphA2 gene was cloned into the eukaryotic CMV promoter-based expression plasmid pCDNA4 (Invitrogen, Carlsbad, CA). Figure 7 shows the results of a Western blot analysis of lystates prepared from 293 cells transfected with the pCDNA4-EphA2 plasmid, and demonstrates the abundant expression in mammalian cells of full-length EphA2 protein.

6.3 EXAMPLE 3: *IN VIVO* EphA2 EFFICACY STUDIES

[00365] Efficacy studies were performed in mice inoculated with CT26 tumor cells expressing the extracellular domain (ED) of human EphA2 in order to characterize the anti-tumor effect of huEphA2. Endpoints measured were tumor volume and percent survival of the mice after tumor inoculation. The routes of inoculation were subcutaneous (s.c.) and intravenous (i.v.). HBSS and *Listeria* were administered as controls.

6.3.1 Therapeutic Vaccinations:

[00366] A representative therapeutic study was performed as follows:

[00367] **Groups:** Six groups of ten mice per group. Groups 1-3 were inoculated s.c. and groups 4-6 were inoculated i.v. with CT26 murine colon carcinoma cells:

Treatment Group	Number of Mice per Groups
1. Control - HBSS	10
2. L4029 – control *Listeria monocytogenes*	10
3. L4029-EphA2 exFlag – *Listeria monocytogenes* expressing extracellular domain of human EphA2	10
4. Control - HBSS	10
5. L4029 – control *Listeria monocytogenes*	10
6. L4029-EphA2 exFlag – *Listeria monocytogenes* expressing extracellular domain of human EphA2	10

[00368] **Schedule:** Animals were inoculated with CT26 colon carcinoma cells transfected with human EphA2 (L4029-EphA2 exFlag), *Listeria* control (L4029-control) or vehicle (HBSS) (5×10^5 cells in 100µl volume) either subcutaneously or intravenously (experimental lung metastases model). Three days after cell inoculation, animals received i.v. administrations of the agents listed above in 200µl bolus. Two weeks following the first administration, the animals received a booster vaccination. Tumor volume was measured bi-weekly (s.c inoculation only) and animal weights assessed on a weekly basis. Any animals possessing tumors greater than 2000 mm^3 or demonstrating signs of morbidity (hunched posture, impaired breathing, decreases mobility, greater than 20% weight loss, *etc.*) were humanely euthanized. The schedule is summarized in the table below.

Group	Cell Inoculation Route (5×10^5 cell in 100µl)	Primary Vaccination (Day 3)	Boost Vaccination (Day 17)
1. Control	s.c.	HBSS	HBSS
2. L4029	s.c.	6×10^6 to 2×10^7 CFU	6×10^6 to 2×10^7 CFU
3. L4029 EphA2-exFlag	s.c.	6×10^6 to 2×10^7 CFU	6×10^6 to 2×10^7 CFU
4. Control	i.v.	HBSS	HBSS
5. L4029	i.v.	6×10^6 to 2×10^7 CFU	6×10^6 to 2×10^7 CFU
6. L4029 EphA2-exFlag	i.v.	6×10^6 to 2×10^7 CFU	6×10^6 to 2×10^7 CFU

[00369] Figures 8A-8C illustrate the results of a typical therapeutic study. In Figure 8A, tumor volume was measured at several intervals post inoculation. Compared to the HBSS and *Listeria* controls, the mice inoculated with CT26 cells expressing the ECD of huEphA2 had a significantly lower tumor volume after day 14 and continued onto day 28. Figure 8B depicts the mean tumor volume of mice inoculated with CT26 cells containing either *Listeria* control or huEphA2. Compared to control, the mice inoculated with CT26 cells expressing huEphA2 had a reduced mean tumor volume. Figure 8C represents the results of the therapeutic study using the lung metastases model, measuring percent survival

of the mice post inoculation with CT26 cells with either HBSS or *Listeria* control, or *Listeria* expressing the ECD of huEphA2. Animals inoculated with CT26 cells expressing the ECD of huEphA2 (depicted by red triangles) showed a higher percent survival rate compared to controls.

6.3.2 Prophylactic Vaccinations:

[00370] Preventive studies were performed according to the schedule described below. These studies utilized a pool of CT26 cells expressing huEphA2 generated by the single cell FACS assays described above.

[00371] **Groups:** Eight groups of ten mice per group. Groups 1-4 were inoculated s.c. and groups 5-8 were inoculated i.v. with CT26 colon carcinoma cells transfected with human EphA2:

Treatment Group	Number of Mice per Groups
1. Control - HBSS	10
2. L4029 – control *Listeria monocytogenes*	10
3. L4029-EphA2 exFlag – *Listeria monocytogenes* expressing extracellular domain of human EphA2	10
4. L4029 – AH1 *Listeria monocytogenes*	10
5. Control - HBSS	10
6. L4029 – control *Listeria monocytogenes*	10
7. L4029-EphA2 exFlag – *Listeria monocytogenes* expressing extracellular domain of human EphA2	10
8. L4029 – AH1 *Listeria monocytogenes*	10

[00372] **Schedule:** Animals received i.v. administrations of the agents listed above in 200µl bolus on Day 0 and Day 10. On Day 14, animals were inoculated with CT26 colon carcinoma cells transfected with human EphA2 (L4029EphA2-exFlag), *Listeria* control (L4029), or *Listeria* positive control containing the AH1 protein (L4029-AH1) (5×10^5 cells in 100µl volume) either subcutaneously or intravenously (experimental lung metastases model). Tumor volume was measured bi-weekly (s.c inoculation only) and animal weights assessed on a weekly basis. Any animals possessing tumors greater than 2000 mm^3 or demonstrating signs of morbidity (hunched posture, impaired breathing, decreases mobility, greater than 20% weight loss, *etc.*) were humanely euthanized. The experimental schedule is summarized in the table below.

Group	Cell Inoculation Route (5×10^5 cell in 100µl) (Day 14)	Primary Vaccination (Day 0)	Boost Vaccination (Day 10)
1. Control	s.c.	HBSS	HBSS
2. L4029	s.c.	2×10^7 CFU	2×10^7 CFU
3. L4029 EphA2-exFlag	s.c.	2×10^7 CFU	2×10^7 CFU

4. L4029 –AH1	s.c.	$2x10^7$ CFU	$2x10^7$ CFU
5. Control	i.v.	HBSS	HBSS
6. L4029	i.v.	$2x10^7$ CFU	$2x10^7$ CFU
7. L4029 EphA2-exFlag	i.v.	$2x10^7$ CFU	$2x10^7$ CFU
8. L4029 – AH1	i.v.	$2x10^7$ CFU	$2x10^7$ CFU

[00373] In this study, vaccination with *Listeria*-huEphA2 exFlag demonstrated a significant anti-tumor effect in both the s.c. and experimental lung metastases models (i.v.). In the s.c. model, a significant reduction in tumor growth was achieved with 3 mice remaining tumor-free. This response was also specific compared to the control *Listeria* and vehicle treated animals. In the experimental lung metastases model, vaccination with *Listeria* huEphA2-exFlag also demonstrated efficacy compared to the vehicle treated group.

[00374] Figures 9A-9D illustrate results of the preventive experiments. Figure 9A shows that the tumor volume of mice inoculated with CT26 cells expressing the ECD of huEphA2 was significantly reduced when compared to vehicle (HBSS), *Listeria* (L4029) and *Listeria* positive (L4029-AH1) controls starting at day 21 and continued until day 32 post inoculation. Figure 9B also depicts results of the preventive experiments, showing again that the tumor volume of mice inoculated with CT26 cells expressing the ECD of huEphA2 (L4029-EphA2 exFlag) was significantly reduced when compared to the *Listeria* (L4029) control starting at day 21 and continued until day 32 post inoculation. Figure 9C illustrates the results of the prevention study in the s.c. model, measuring percent survival of the mice post CT26 tumor cell inoculation. Compared to all control groups, the L4029-EphA2 exFlag group had the most significant survival rate (indicated by red triangles). Figure 9D illustrates the results of the prevention study in the lung metastases model, measuring the percent survival of the mice post tumor cell inoculation. Compared to all control groups, the L4029-EphA2 exFlag group had the most significant survival rate.

6.4 Therapeutic efficacy in Balb/C mice bearing CT26 tumors encoding human EphA2 immunized with recombinant *Listeria* encoding codon-optimized EphA2

[00375] The following data presented in Figures 10-13 demonstrated the following:

[00376] Immunization of Balb/C mice bearing CT26.24 (huEphA2+) lung tumors with recombinant *Listeria* encoding OVA.AH1 (MMTV gp70 immunodominant epitope) or OVA.AH1-A5 (MMTV gp70 immunodominant epitope, with heteroclitic change for enhanced T-cell receptor binding) confers long-term survival (Figure 10).

[00377] The EphA2 CO domain is strongly immunogenic, and a significant long term increase in survival of Balb/C mice bearing CT26.24 (huEphA2+) lung tumors was

observed when immunized with recombinant *Listeria* encoding codon-optimized or native EphA2 CO domain sequence (Figure 12).

[00378] The EphA2 EX2 domain is poorly immunogenic, and increased survival of Balb/C mice bearing CT26.24 (huEphA2+) lung tumors was observed only when immunized with recombinant *Listeria* encoding codon-optimized secA1 signal peptide fused with the codon-optimized EphA2 EX2 domain sequence. Therapeutic efficacy was not observed in mice when immunized with recombinant *Listeria* encoding native secA1 signal peptide fused with the codon-optimized EphA2 EX2 domain sequence (Figure 11). The desirability of using both codon-optimized secA1 signal peptide and EphA2 EX2 domain sequences was supported by statistically significant therapeutic anti-tumor efficacy, as shown in the table below:

[00379] The following table shows a comparison by log-rank test of survival curves shown in Figure 11:

Experimental Group	Median Survival (Days)	Significance versus HBSS cohort (p value)	Significance versus actA-native secA1/EphA2 EX2 cohort (p value)
HBSS	19	-	-
ActA	20	NS	NS
actA-native secA1-EphA2 EX2 (native)	19	NS	-
actA-native secA1-EphA2 EX2 (CodOp)	24	0.0035	NS
actA-CodOp secA1-EphA2 EX2 (CodOp)	37	0.0035	0.0162
actA-native secA1-EphA2 CO (CodOp)	>99	0.0035	0.0015

[00380] Significantly, even though pCDNA4-EphA2 plasmid transfected 293 cells yielded very high levels of protein expression, immunization of Balb/C mice bearing CT26.24 (huEphA2+) lung tumors with the pCDNA4-EphA2 plasmid did not result in any observance of therapeutic anti-tumor efficacy (Figure 13).

[00381] For therapeutic *in vivo* tumor studies, female Balb/C mice were implanted IV with 5 x 10^5 CT26 cells stably expressing EphA2. Three days later, mice were randomized and vaccinated IV with various recombinant *Listeria* strains encoding EphA2. In some cases (noted in figures) mice were vaccinated with 100 μg of pCDNA4 plasmid or pCDNA4-EphA2 plasmid in the *tibialis* anterior muscle. As a positive control, mice were vaccinated IV with recombinant *Listeria* strains encoding OVA.AHI or OVA.AH1-A5 protein chimeras. Mice were vaccinated on days 3 and 14 following tumor cell implantation. Mice injected with Hanks Balanced Salt Solution (HBSS) buffer or unmodified *Listeria* served as negative controls. All experimental cohorts contained 5

mice. For survival studies mice were sacrificed when they started to show any signs of stress or labored breathing.

7. EQUIVALENTS

[00382] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

[00383] All publications, patents and patent applications mentioned in this specification are herein incorporated by reference into the specification to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference.

We claim:

1. A method of eliciting an immune response against an EphA2-expressing cell, said method comprising administering to an individual a composition comprising an EphA2 antigenic polypeptide in an amount effective to elicit an immune response against an EphA2-expressing cell.

2. The method of claim 1, wherein the composition further comprises an adjuvant.

3. The method of claim 1, wherein the composition comprises a heat shock protein bound to said EphA2 antigenic polypeptide.

4. The method of claim 1, where the polypeptide further comprises a protein transduction domain.

5. The method of claim 4, wherein the protein transduction domain is the Antennapedia or the HIV tat protein transduction domain.

6. A method of eliciting an immune response against an EphA2-expressing cell, said method comprising administering to an individual a composition comprising an EphA2 antigenic polypeptide expression vehicle in an amount effective to elicit an immune response against an EphA2-expressing cell.

7. The method of claim 6, wherein the expression vehicle is a nucleic acid encoding said EphA2 antigenic polypeptide operably linked to a promoter.

8. The method of claim 7, wherein the nucleic acid is DNA.

9. The method of claim 8, wherein the DNA is conjugated to a carrier.

10. The method of claim 7, wherein the carrier is asialoglycoprotein.

11. The method of claim 7, wherein the carrier is transferrin.

12. The method of claim 7, wherein the carrier is polymeric IgA.

13. The method of claim 6, wherein the expression vehicle is an infectious agent comprising a nucleic acid, said nucleic acid comprising a nucleotide sequence encoding said EphA2 antigenic polypeptide operably linked to a promoter.

14. The method of claim 13, wherein the sequence encoding said EphA2 antigenic polypeptide is codon-optimized for expression in said infectious agent.

15. The method of claim 13, wherein the infectious agent is coated with a reagent that targets the infectious agent to EphA2-expressing cells.

16. The method of claim 15, wherein the reagent is an anti-EphA2 antibody.

17. The method of claim 13, wherein the infectious agent is coated with a reagent that targets the infectious agent to antigen-presenting cells.

18. The method of claim 13, wherein the infectious agent is a bacterium.

19. The method of claim 18, wherein the bacterium is attenuated.

20. The method of claim 19, wherein the attenuated bacterium is deficient in DNA repair.

21. The method of claim 19, wherein the bacterium is psoralen-treated.

22. The method of claim 20, wherein the bacterium has a mutation in a DNA repair gene.

23. The method of claim 18, wherein the nucleic acid comprises a nucleotide sequence encoding a secretory signal operatively linked to the sequence encoding the EphA2 antigenic polypeptide.

24. The method of claim 23, wherein the secretory signal is a SecA secretory signal.

25. The method of claim 18, wherein the bacterium is *Pseudomonas aeruginosa*.

26. The method of claim 13, wherein the infectious agent is a virus.

27. The method of claim 26, wherein the virus is a retrovirus.

28. The method of claim 27, wherein the retrovirus is a lentivirus.

29. The method of claim 26, wherein the virus is an adenovirus.

30. The method of claim 26, wherein the virus is an adeno-associated virus.

31. The method of claim 26, wherein the virus is herpes simplex virus.

32. The method of claim 26, wherein the virus is attenuated.

33. The method of claim 6, wherein the expression vehicle is a mammalian cell comprising a recombinant nucleic acid, said nucleic acid comprising a nucleotide sequence encoding said EphA2 antigenic polypeptide.

34. The method of claim 34, wherein the mammalian cell is a human cell.

35. The method of claim 33, wherein the mammalian cell is encapsulated within a membrane.

36. The method of claim 35, wherein said administering is by means of implantation.

37. A method of eliciting an immune response against an EphA2-expressing cell, said method comprising administering to an individual a composition comprising antigen presenting cells sensitized with an EphA2 antigenic polypeptide.

38. The method of claim 37, further comprising prior to said administration the step of sensitizing the antigen presenting cells.

39. The method of claim 38, wherein the antigen presenting cells are sensitized by a method comprising: contacting the cells with a composition comprising one or more EphA2 antigenic peptides in an amount effective to sensitize the cells.

40. The method of claim 40, wherein the composition further comprises a heat shock protein.

41. The method of claim 40, wherein the heat shock protein is hsp70, gp96, or hsp90.

42. The method of claim 37, wherein the antigen presenting cells are autologous to the individual.

43. The method of claim 37, wherein the antigen presenting cells are non-autologous to the individual.

44. The method of claim 37, wherein the antigen presenting cells are macrophages.

45. The method of claim 37, wherein the antigen presenting cells are dendritic cells.

46. The method of claim 1, 6, or 37, wherein the individual has cancer.

47. The method of claim 46, wherein said cancer is of an epithelial cell origin.

48. The method of claim 46, wherein said cancer comprises cells that overexpress EphA2 relative to non-cancer cells having the tissue type of said cancer cells.

49. The method of claim 46, wherein said cancer is a cancer of the skin, lung, colon, ovary, esophagus, breast, prostate, bladder or pancreas or is a renal cell carcinoma or melanoma.

50. The method of claim 1, 6, 37, or 53, wherein the individual has a non-neoplastic hyperproliferative disorder.

51. The method of claim 50, wherein the hyperproliferative disorder is an epithelial cell disorder.

52. The method of claim 51, wherein the hyperproliferative is asthma, chronic pulmonary obstructive disease, lung fibrosis, bronchial hyper responsiveness, psoriasis, and seborrheic dermatitis.

53. A method of eliciting an immune response against an EphA2-expressing cell, said method comprising administering to an individual a composition comprising an anti-idiotypic antibody or antigen-binding fragment thereof which immunospecifically binds to an idiotype of an anti-EphA2 antibody in an amount effective to elicit an immune response against an EphA2-expressing cell.

54. A method of treating a human individual having a hyperproliferative disorder of EphA2-expressing cells, said method comprising administering to the individual a composition comprising an EphA2 antigenic polypeptide in an amount effective to treat a hyperproliferative disorder of EphA2-expressing cells.

55. A method of treating a human individual having a hyperproliferative disorder of EphA2-expressing cells, said method comprising administering to the individual a composition comprising an EphA2 expression vehicle in an amount effective to treat a hyperproliferative disorder of EphA2-expressing cells.

56. A method of treating a human individual having a hyperproliferative disorder of EphA2-expressing cells, said method comprising administering to the individual a composition comprising antigen presenting cells sensitized with an EphA2 antigenic polypeptide in an amount effective to treat a hyperproliferative disorder of EphA2-expressing cells.

57. A method of treating a human individual having a hyperproliferative disorder of EphA2-expressing cells, said method comprising administering to an individual a composition comprising an anti-idiotypic antibody or antigen-binding fragment thereof which immunospecifically binds to an idiotype of an anti-EphA2 antibody in an amount effective to elicit treat a hyperproliferative disorder of EphA2-expressing cells.

58. The method of claim 54, 55, 56, or 57, wherein the individual has cancer.

59. The method of claim 58, wherein said cancer is of an epithelial cell origin.

60. The method of claim 58, wherein said cancer comprises cells that overexpress EphA2 relative to non-cancer cells having the tissue type of said cancer cells.

61. The method of claim 58, wherein said cancer is a cancer of the skin, lung, colon, breast, prostate, bladder or pancreas or is a renal cell carcinoma or melanoma.

62. The method of claim 54, 55, 56, or 57, wherein the individual has a non-neoplastic hyperproliferative disorder.

63. The method of claim 62, wherein the hyperproliferative disorder is an epithelial cell disorder.

64. The method of claim 63, wherein the hyperproliferative disorder is asthma, chronic pulmonary obstructive disease, lung fibrosis, bronchial hyper responsiveness, psoriasis, and seborrheic dermatitis.

65. The method of any one of claims 1, 6, 37, 54, 55, and 56, wherein the EphA2 polypeptide comprises full length EphA2.

66. The method of any one of claims 1, 6, 37, 54, 55, and 56, wherein the EphA2 polypeptide comprises the extracellular domain of EphA2.

67. The method of any one of claims 1, 6, 37, 54, 55, and 56, wherein the EphA2 polypeptide comprises the intracellular domain of EphA2.

68. The method of any one of claims 1, 6, 37, 54, 55, and 56, wherein the EphA2 polypeptide is a chimeric polypeptide comprising at least an antigenic portion of EphA2 and a second polypeptide.

69. The method of claim 53 or 57, wherein the EphA2 antibody immunospecificallybinds to an epitope in the extracellular domain of EphA2.

70. The method of claim 53 or 57, wherein the EphA2 antibody immunospecificallybinds to an epitope the intracellular domain of EphA2.

71. The method of any one of claims 1, 6, 37, 54, 55, and 56, wherein the EphA2 polypeptide is a chimeric polypeptide comprising at least an antigenic portion of EphA2 and a second polypeptide.

72. The method of claim 1, or 54, wherein the composition comprises a plurality of EphA2 antigenic polypeptides.

73. The method of claim 6 or 55, wherein the composition comprises a plurality of EphA2 antigenic polypeptide expression vehicles.

74. The method of claim 6 or 55, wherein the expression vehicle expresses a plurality of EphA2 antigenic polypeptides.

75. The method of claim 37 or 56, wherein the antigen presenting cells are sensitized with a plurality of EphA2 antigenic polypeptides

76. The method of any one of claims 1, 6, 37, 53, 54, 55, 56, and 57, further comprising administering an additional anti-cancer therapy.

77. The method of claim 76, wherein the additional anti-cancer therapy is an agonistic EphA2 antibody.

78. The method of claim 76, wherein the additional anti-cancer therapy is chemotherapy, biological therapy, immunotherapy, radiation therapy, hormonal therapy, or surgery.

79. The method of any one of claims 1, 6, 37, 53, 54, 55, 56, and 57, wherein said administering is mucosal, intranasal, parenteral, intramuscular, or intraperitoneal.

ABSTRACT

[00384] The present invention relates to methods and compositions designed for the treatment, management, or prevention of cancer, particularly metastatic cancer, and hyperproliferative diseases involving EphA2-expressing cells. In one embodiment, the methods of the invention comprise the administration of an effective amount of an EphA2 antigenic peptide to elicit an immune response against the EphA2-expressing cells. In other embodiments, the methods of the invention entail the use of an EphA2 expression vehicle, such as a naked nucleic acid or viral vector. In yet other embodiments, the methods of the invention comprise the use of adoptive immunotherapy with autologous or non-autologous antigen presenting cells that are sensitized with one or more EphA2 antigenic peptides. The invention also provides pharmaceutical compositions comprising one or more EphA2 antigenic peptides, expression vehicles or antigen-presenting cells of the invention either alone or in combination with one or more other agents useful for cancer therapy.

SEQUENCE LISTING

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Val Pro Pro Gly Gly Glu Glu Pro Arg Met His Cys Ala Val Asp Gly
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gag tgg ctg gtg ccc att ggg cag tgc ctg tgc cag gca ggc tac gag      938
Glu Trp Leu Val Pro Ile Gly Gln Cys Leu Cys Gln Ala Gly Tyr Glu
            255                 260                 265

aag gtg gag gat gcc tgc cag gcc tgc tcg cct gga ttt ttt aag ttt      986
Lys Val Glu Asp Ala Cys Gln Ala Cys Ser Pro Gly Phe Phe Lys Phe
        270                 275                 280

gag gca tct gag agc ccc tgc ttg gag tgc cct gag cac acg ctg cca     1034
Glu Ala Ser Glu Ser Pro Cys Leu Glu Cys Pro Glu His Thr Leu Pro
    285                 290                 295

tcc cct gag ggt gcc acc tcc tgc gag tgt gag gaa ggc ttc ttc cgg     1082
Ser Pro Glu Gly Ala Thr Ser Cys Glu Cys Glu Glu Gly Phe Phe Arg
300                 305                 310                 315

gca cct cag gac cca gcg tcg atg cct tgc aca cga ccc ccc tcc gcc     1130
Ala Pro Gln Asp Pro Ala Ser Met Pro Cys Thr Arg Pro Pro Ser Ala
                320                 325                 330

cca cac tac ctc aca gcc gtg ggc atg ggt gcc aag gtg gag ctg cgc     1178
Pro His Tyr Leu Thr Ala Val Gly Met Gly Ala Lys Val Glu Leu Arg
            335                 340                 345

tgg acg ccc cct cag gac agc ggg ggc cgc gag gac att gtc tac agc     1226
Trp Thr Pro Pro Gln Asp Ser Gly Gly Arg Glu Asp Ile Val Tyr Ser
        350                 355                 360

gtc acc tgc gaa cag tgc tgg ccc gag tct ggg gaa tgc ggg ccg tgt     1274
Val Thr Cys Glu Gln Cys Trp Pro Glu Ser Gly Glu Cys Gly Pro Cys
    365                 370                 375
```

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gag gcc agt gtg cgc tac tcg gag cct cct cac gga ctg acc cgc acc      1322
Glu Ala Ser Val Arg Tyr Ser Glu Pro Pro His Gly Leu Thr Arg Thr
380                 385                 390                 395

agt gtg aca gtg agc gac ctg gag ccc cac atg aac tac acc ttc acc      1370
Ser Val Thr Val Ser Asp Leu Glu Pro His Met Asn Tyr Thr Phe Thr
                400                 405                 410

gtg gag gcc cgc aat ggc gtc tca ggc ctg gta acc agc cgc agc ttc      1418
Val Glu Ala Arg Asn Gly Val Ser Gly Leu Val Thr Ser Arg Ser Phe
            415                 420                 425

cgt act gcc agt gtc agc atc aac cag aca gag ccc ccc aag gtg agg      1466
Arg Thr Ala Ser Val Ser Ile Asn Gln Thr Glu Pro Pro Lys Val Arg
        430                 435                 440

ctg gag ggc cgc agc acc acc tcg ctt agc gtc tcc tgg agc atc ccc      1514
Leu Glu Gly Arg Ser Thr Thr Ser Leu Ser Val Ser Trp Ser Ile Pro
    445                 450                 455

ccg ccg cag cag agc cga gtg tgg aag tac gag gtc act tac cgc aag      1562
Pro Pro Gln Gln Ser Arg Val Trp Lys Tyr Glu Val Thr Tyr Arg Lys
460                 465                 470                 475

aag gga gac tcc aac agc tac aat gtg cgc cgc acc gag ggt ttc tcc      1610
Lys Gly Asp Ser Asn Ser Tyr Asn Val Arg Arg Thr Glu Gly Phe Ser
                480                 485                 490

gtg acc ctg gac gac ctg gcc cca gac acc acc tac ctg gtc cag gtg      1658
Val Thr Leu Asp Asp Leu Ala Pro Asp Thr Thr Tyr Leu Val Gln Val
            495                 500                 505

cag gca ctg acg cag gag ggc cag ggg gcc ggc agc aag gtg cac gaa      1706
Gln Ala Leu Thr Gln Glu Gly Gln Gly Ala Gly Ser Lys Val His Glu
        510                 515                 520

ttc cag acg ctg tcc ccg gag gga tct ggc aac ttg gcg gtg att ggc      1754
Phe Gln Thr Leu Ser Pro Glu Gly Ser Gly Asn Leu Ala Val Ile Gly
    525                 530                 535

ggc gtg gct gtc ggt gtg gtc ctg ctt ctg gtg ctg gca gga gtt ggc      1802
Gly Val Ala Val Gly Val Val Leu Leu Leu Val Leu Ala Gly Val Gly
540                 545                 550                 555

ttc ttt atc cac cgc agg agg aag aac cag cgt gcc cgc cag tcc ccg      1850
Phe Phe Ile His Arg Arg Arg Lys Asn Gln Arg Ala Arg Gln Ser Pro
                560                 565                 570

gag gac gtt tac ttc tcc aag tca gaa caa ctg aag ccc ctg aag aca      1898
Glu Asp Val Tyr Phe Ser Lys Ser Glu Gln Leu Lys Pro Leu Lys Thr
            575                 580                 585

tac gtg gac ccc cac aca tat gag gac ccc aac cag gct gtg ttg aag      1946
Tyr Val Asp Pro His Thr Tyr Glu Asp Pro Asn Gln Ala Val Leu Lys
        590                 595                 600

ttc act acc gag atc cat cca tcc tgt gtc act cgg cag aag gtg atc      1994
Phe Thr Thr Glu Ile His Pro Ser Cys Val Thr Arg Gln Lys Val Ile
    605                 610                 615
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gga gca gga gag ttt ggg gag gtg tac aag ggc atg ctg aag aca tcc     2042
Gly Ala Gly Glu Phe Gly Glu Val Tyr Lys Gly Met Leu Lys Thr Ser
620                 625                 630                 635

tcg ggg aag aag gag gtg ccg gtg gcc atc aag acg ctg aaa gcc ggc     2090
Ser Gly Lys Lys Glu Val Pro Val Ala Ile Lys Thr Leu Lys Ala Gly
                640                 645                 650

tac aca gag aag cag cga gtg gac ttc ctc ggc gag gcc ggc atc atg     2138
Tyr Thr Glu Lys Gln Arg Val Asp Phe Leu Gly Glu Ala Gly Ile Met
            655                 660                 665

ggc cag ttc agc cac cac aac atc atc cgc cta gag ggc gtc atc tcc     2186
Gly Gln Phe Ser His His Asn Ile Ile Arg Leu Glu Gly Val Ile Ser
        670                 675                 680

aaa tac aag ccc atg atg atc atc act gag tac atg gag aat ggg gcc     2234
Lys Tyr Lys Pro Met Met Ile Ile Thr Glu Tyr Met Glu Asn Gly Ala
    685                 690                 695

ctg gac aag ttc ctt cgg gag aag gat ggc gag ttc agc gtg ctg cag     2282
Leu Asp Lys Phe Leu Arg Glu Lys Asp Gly Glu Phe Ser Val Leu Gln
700                 705                 710                 715

ctg gtg ggc atg ctg cgg ggc atc gca gct ggc atg aag tac ctg gcc     2330
Leu Val Gly Met Leu Arg Gly Ile Ala Ala Gly Met Lys Tyr Leu Ala
                720                 725                 730

aac atg aac tat gtg cac cgt gac ctg gct gcc cgc aac atc ctc gtc     2378
Asn Met Asn Tyr Val His Arg Asp Leu Ala Ala Arg Asn Ile Leu Val
            735                 740                 745

aac agc aac ctg gtc tgc aag gtg tct gac ttt ggc ctg tcc cgc gtg     2426
Asn Ser Asn Leu Val Cys Lys Val Ser Asp Phe Gly Leu Ser Arg Val
        750                 755                 760

ctg gag gac gac ccc gag gcc acc tac acc acc agt ggc ggc aag atc     2474
Leu Glu Asp Asp Pro Glu Ala Thr Tyr Thr Thr Ser Gly Gly Lys Ile
    765                 770                 775

ccc atc cgc tgg acc gcc ccg gag gcc att tcc tac cgg aag ttc acc     2522
Pro Ile Arg Trp Thr Ala Pro Glu Ala Ile Ser Tyr Arg Lys Phe Thr
780                 785                 790                 795

tct gcc agc gac gtg tgg agc ttt ggc att gtc atg tgg gag gtg atg     2570
Ser Ala Ser Asp Val Trp Ser Phe Gly Ile Val Met Trp Glu Val Met
                800                 805                 810

acc tat ggc gag cgg ccc tac tgg gag ttg tcc aac cac gag gtg atg     2618
Thr Tyr Gly Glu Arg Pro Tyr Trp Glu Leu Ser Asn His Glu Val Met
            815                 820                 825

aaa gcc atc aat gat ggc ttc cgg ctc ccc aca ccc atg gac tgc ccc     2666
Lys Ala Ile Asn Asp Gly Phe Arg Leu Pro Thr Pro Met Asp Cys Pro
        830                 835                 840

tcc gcc atc tac cag ctc atg atg cag tgc tgg cag cag gag cgt gcc     2714
Ser Ala Ile Tyr Gln Leu Met Met Gln Cys Trp Gln Gln Glu Arg Ala
    845                 850                 855

cgc cgc ccc aag ttc gct gac atc gtc agc atc ctg gac aag ctc att     2762
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Arg Arg Pro Lys Phe Ala Asp Ile Val Ser Ile Leu Asp Lys Leu Ile
860                 865                 870                 875

cgt gcc cct gac tcc ctc aag acc ctg gct gac ttt gac ccc cgc gtg     2810
Arg Ala Pro Asp Ser Leu Lys Thr Leu Ala Asp Phe Asp Pro Arg Val
                880                 885                 890

tct atc cgg ctc ccc agc acg agc ggc tcg gag ggg gtg ccc ttc cgc     2858
Ser Ile Arg Leu Pro Ser Thr Ser Gly Ser Glu Gly Val Pro Phe Arg
            895                 900                 905

acg gtg tcc gag tgg ctg gag tcc atc aag atg cag cag tat acg gag     2906
Thr Val Ser Glu Trp Leu Glu Ser Ile Lys Met Gln Gln Tyr Thr Glu
        910                 915                 920

cac ttc atg gcg gcc ggc tac act gcc atc gag aag gtg gtg cag atg     2954
His Phe Met Ala Ala Gly Tyr Thr Ala Ile Glu Lys Val Val Gln Met
    925                 930                 935

acc aac gac gac atc aag agg att ggg gtg cgg ctg ccc ggc cac cag     3002
Thr Asn Asp Asp Ile Lys Arg Ile Gly Val Arg Leu Pro Gly His Gln
940                 945                 950                 955

aag cgc atc gcc tac agc ctg ctg gga ctc aag gac cag gtg aac act     3050
Lys Arg Ile Ala Tyr Ser Leu Leu Gly Leu Lys Asp Gln Val Asn Thr
                960                 965                 970

gtg ggg atc ccc atc tga gcctcgacag ggcctggagc cccatcggcc            3098
Val Gly Ile Pro Ile
            975

aagaatactt gaagaaacag agtggcctcc ctgctgtgcc atgctgggcc actggggact   3158

ttatttattt ctagttcttt cctccccctg caacttccgc tgaggggtct cggatgacac   3218

cctggcctga actgaggaga tgaccaggga tgctgggctg ggccctcttt ccctgcgaga   3278

cgcacacagc tgagcactta gcaggcaccg ccacgtccca gcatccctgg agcaggagcc   3338

ccgccacagc cttcggacag acatatagga tattcccaag ccgaccttcc ctccgccttc   3398

tcccacatga ggccatctca ggagatggag ggcttggccc agcgccaagt aaacagggta   3458

cctcaagccc catttcctca cactaagagg gcagactgtg aacttgactg ggtgagaccc   3518

aaagcggtcc ctgtccctct agtgccttct ttagaccctc gggccccatc ctcatccctg   3578

actggccaaa cccttgcttt cctgggcctt tgcaagatgc ttggttgtgt tgaggttttt   3638

aaatatatat tttgtacttt gtggagagaa tgtgtgtgtg tggcaggggg ccccgccagg   3698

gctggggaca gagggtgtca aacattcgtg agctggggac tcagggaccg gtgctgcagg   3758

agtgtcctgc ccatgcccca gtcggcccca tctctcatcc ttttggataa gtttctattc   3818

tgtcagtgtt aaagattttg ttttgttgga catttttttc gaatcttaat ttattatttt   3878

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caaaccgaaa aaaaaaaaaa aaaaa                                        3963
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Met Glu Leu Gln Ala Ala Arg Ala Cys Phe Ala Leu Leu Trp Gly Cys
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Ala Leu Ala Ala Ala Ala Ala Ala Gln Gly Lys Glu Val Val Leu Leu
            20                  25                  30

Asp Phe Ala Ala Ala Gly Gly Glu Leu Gly Trp Leu Thr His Pro Tyr
        35                  40                  45

Gly Lys Gly Trp Asp Leu Met Gln Asn Ile Met Asn Asp Met Pro Ile
    50                  55                  60

Tyr Met Tyr Ser Val Cys Asn Val Met Ser Gly Asp Gln Asp Asn Trp
65                  70                  75                  80

Leu Arg Thr Asn Trp Val Tyr Arg Gly Glu Ala Glu Arg Ile Phe Ile
                85                  90                  95

Glu Leu Lys Phe Thr Val Arg Asp Cys Asn Ser Phe Pro Gly Gly Ala
            100                 105                 110

Ser Ser Cys Lys Glu Thr Phe Asn Leu Tyr Tyr Ala Glu Ser Asp Leu
        115                 120                 125

Asp Tyr Gly Thr Asn Phe Gln Lys Arg Leu Phe Thr Lys Ile Asp Thr
    130                 135                 140

Ile Ala Pro Asp Glu Ile Thr Val Ser Ser Asp Phe Glu Ala Arg His
145                 150                 155                 160

Val Lys Leu Asn Val Glu Glu Arg Ser Val Gly Pro Leu Thr Arg Lys
                165                 170                 175

Gly Phe Tyr Leu Ala Phe Gln Asp Ile Gly Ala Cys Val Ala Leu Leu
            180                 185                 190

Ser Val Arg Val Tyr Tyr Lys Lys Cys Pro Glu Leu Leu Gln Gly Leu
        195                 200                 205

Ala His Phe Pro Glu Thr Ile Ala Gly Ser Asp Ala Pro Ser Leu Ala
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```
    210                 215                 220

Thr Val Ala Gly Thr Cys Val Asp His Ala Val Val Pro Pro Gly Gly
225                 230                 235                 240

Glu Glu Pro Arg Met His Cys Ala Val Asp Gly Glu Trp Leu Val Pro
                245                 250                 255

Ile Gly Gln Cys Leu Cys Gln Ala Gly Tyr Glu Lys Val Glu Asp Ala
            260                 265                 270

Cys Gln Ala Cys Ser Pro Gly Phe Phe Lys Phe Glu Ala Ser Glu Ser
        275                 280                 285

Pro Cys Leu Glu Cys Pro Glu His Thr Leu Pro Ser Pro Glu Gly Ala
    290                 295                 300

Thr Ser Cys Glu Cys Glu Glu Gly Phe Phe Arg Ala Pro Gln Asp Pro
305                 310                 315                 320

Ala Ser Met Pro Cys Thr Arg Pro Pro Ser Ala Pro His Tyr Leu Thr
                325                 330                 335

Ala Val Gly Met Gly Ala Lys Val Glu Leu Arg Trp Thr Pro Pro Gln
            340                 345                 350

Asp Ser Gly Gly Arg Glu Asp Ile Val Tyr Ser Val Thr Cys Glu Gln
        355                 360                 365

Cys Trp Pro Glu Ser Gly Glu Cys Gly Pro Cys Glu Ala Ser Val Arg
    370                 375                 380

Tyr Ser Glu Pro Pro His Gly Leu Thr Arg Thr Ser Val Thr Val Ser
385                 390                 395                 400

Asp Leu Glu Pro His Met Asn Tyr Thr Phe Thr Val Glu Ala Arg Asn
                405                 410                 415

Gly Val Ser Gly Leu Val Thr Ser Arg Ser Phe Arg Thr Ala Ser Val
            420                 425                 430

Ser Ile Asn Gln Thr Glu Pro Pro Lys Val Arg Leu Glu Gly Arg Ser
        435                 440                 445

Thr Thr Ser Leu Ser Val Ser Trp Ser Ile Pro Pro Pro Gln Gln Ser
    450                 455                 460
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Arg Val Trp Lys Tyr Glu Val Thr Tyr Arg Lys Lys Gly Asp Ser Asn
465                 470                 475                 480

Ser Tyr Asn Val Arg Arg Thr Glu Gly Phe Ser Val Thr Leu Asp Asp
                485                 490                 495

Leu Ala Pro Asp Thr Thr Tyr Leu Val Gln Val Gln Ala Leu Thr Gln
            500                 505                 510

Glu Gly Gln Gly Ala Gly Ser Lys Val His Glu Phe Gln Thr Leu Ser
        515                 520                 525

Pro Glu Gly Ser Gly Asn Leu Ala Val Ile Gly Gly Val Ala Val Gly
    530                 535                 540

Val Val Leu Leu Leu Val Leu Ala Gly Val Gly Phe Phe Ile His Arg
545                 550                 555                 560

Arg Arg Lys Asn Gln Arg Ala Arg Gln Ser Pro Glu Asp Val Tyr Phe
                565                 570                 575

Ser Lys Ser Glu Gln Leu Lys Pro Leu Lys Thr Tyr Val Asp Pro His
            580                 585                 590

Thr Tyr Glu Asp Pro Asn Gln Ala Val Leu Lys Phe Thr Thr Glu Ile
        595                 600                 605

His Pro Ser Cys Val Thr Arg Gln Lys Val Ile Gly Ala Gly Glu Phe
    610                 615                 620

Gly Glu Val Tyr Lys Gly Met Leu Lys Thr Ser Ser Gly Lys Lys Glu
625                 630                 635                 640

Val Pro Val Ala Ile Lys Thr Leu Lys Ala Gly Tyr Thr Glu Lys Gln
                645                 650                 655

Arg Val Asp Phe Leu Gly Glu Ala Gly Ile Met Gly Gln Phe Ser His
            660                 665                 670

His Asn Ile Ile Arg Leu Glu Gly Val Ile Ser Lys Tyr Lys Pro Met
        675                 680                 685

Met Ile Ile Thr Glu Tyr Met Glu Asn Gly Ala Leu Asp Lys Phe Leu
    690                 695                 700
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Arg Glu Lys Asp Gly Glu Phe Ser Val Leu Gln Leu Val Gly Met Leu
705                 710                 715                 720

Arg Gly Ile Ala Ala Gly Met Lys Tyr Leu Ala Asn Met Asn Tyr Val
                725                 730                 735

His Arg Asp Leu Ala Ala Arg Asn Ile Leu Val Asn Ser Asn Leu Val
            740                 745                 750

Cys Lys Val Ser Asp Phe Gly Leu Ser Arg Val Leu Glu Asp Asp Pro
        755                 760                 765

Glu Ala Thr Tyr Thr Thr Ser Gly Gly Lys Ile Pro Ile Arg Trp Thr
    770                 775                 780

Ala Pro Glu Ala Ile Ser Tyr Arg Lys Phe Thr Ser Ala Ser Asp Val
785                 790                 795                 800

Trp Ser Phe Gly Ile Val Met Trp Glu Val Met Thr Tyr Gly Glu Arg
                805                 810                 815

Pro Tyr Trp Glu Leu Ser Asn His Glu Val Met Lys Ala Ile Asn Asp
            820                 825                 830

Gly Phe Arg Leu Pro Thr Pro Met Asp Cys Pro Ser Ala Ile Tyr Gln
        835                 840                 845

Leu Met Met Gln Cys Trp Gln Gln Glu Arg Ala Arg Arg Pro Lys Phe
    850                 855                 860

Ala Asp Ile Val Ser Ile Leu Asp Lys Leu Ile Arg Ala Pro Asp Ser
865                 870                 875                 880

Leu Lys Thr Leu Ala Asp Phe Asp Pro Arg Val Ser Ile Arg Leu Pro
                885                 890                 895

Ser Thr Ser Gly Ser Glu Gly Val Pro Phe Arg Thr Val Ser Glu Trp
            900                 905                 910

Leu Glu Ser Ile Lys Met Gln Gln Tyr Thr Glu His Phe Met Ala Ala
        915                 920                 925

Gly Tyr Thr Ala Ile Glu Lys Val Val Gln Met Thr Asn Asp Asp Ile
    930                 935                 940
```

```
Lys Arg Ile Gly Val Arg Leu Pro Gly His Gln Lys Arg Ile Ala Tyr
945                 950                 955                 960

Ser Leu Leu Gly Leu Lys Asp Gln Val Asn Thr Val Gly Ile Pro Ile
                965                 970                 975


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Thr Leu Ala Asp Phe Asp Pro Arg Val Pro Arg Thr
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Val Leu Leu Leu Val Leu Ala Gly Val
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Val Leu Ala Gly Val Gly Phe Phe Ile
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Ile Met Asn Asp Met Pro Ile Tyr Met
1               5


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Ser Leu Leu Gly Leu Lys Asp Gln Val
1               5
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Trp Leu Val Pro Ile Gly Gln Cys Leu
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Gly Leu Thr Arg Thr Ser Val Thr Val
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Asn Leu Tyr Tyr Ala Glu Ser Asp Leu
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Lys Leu Asn Val Glu Glu Arg Ser Val
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Ile Met Gly Gln Phe Ser His His Asn
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Tyr Ser Val Cys Asn Val Met Ser Gly
1 5

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Met Gln Asn Ile Met Asn Asp Met Pro
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Glu Ala Gly Ile Met Gly Gln Phe Ser His His Asn Ile Ile Arg
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Pro Ile Tyr Met Tyr Ser Val Cys Asn Val Met Ser Gly
1 5 10

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Asp Leu Met Gln Asn Ile Met Asn Asp Met Pro Ile Tyr Met Tyr Ser
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ccaccagcat ctccgcctgc aagtcctaag acgccaatcg aaaagaaaca cgcggatctc 180

gagctccagg cagcccgcgc ctgcttcgcc ctgctgtggg gctgtgcgct ggccgcggcc 240

gcggcggcgc agggcaagga agtggtactg ctggactttg ctgcagctgg aggggagctc 300

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cgcaccaact gggtgtaccg aggagaggct gagcgtatct tcattgagct caagtttact 480

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aagaccctgg ctgactttga cccccgcgtg tctatccggc tccccagcac gagcggctcg 2880

gaggggtgc ccttccgcac ggtgtccgag tggctggagt ccatcaagat gcagcagtat 2940

acggagcact tcatggcggc cggctacact gccatcgaga aggtggtgca gatgaccaac 3000

gacgacatca agaggattgg ggtgcggctg cccggccacc agaagcgcat cgcctacagc 3060

ctgctgggac tcaaggacca ggtgaacact gtggggatcc ccatc 3105

<210> 20
<211> 1035
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Predicted fusion protein

<400> 20

```
Met Lys Lys Ile Met Leu Val Phe Ile Thr Leu Ile Leu Val Ser Leu
1               5                  10                  15

Pro Ile Ala Gln Gln Thr Glu Ala Lys Asp Ala Ser Ala Phe Asn Lys
            20                  25                  30

Glu Asn Ser Ile Ser Ser Met Ala Pro Pro Ala Ser Pro Pro Ala Ser
        35                  40                  45

Pro Lys Thr Pro Ile Glu Lys Lys His Ala Asp Leu Glu Leu Gln Ala
    50                  55                  60

Ala Arg Ala Cys Phe Ala Leu Leu Trp Gly Cys Ala Leu Ala Ala Ala
65                  70                  75                  80

Ala Ala Ala Gln Gly Lys Glu Val Val Leu Leu Asp Phe Ala Ala Ala
                85                  90                  95

Gly Gly Glu Leu Gly Trp Leu Thr His Pro Tyr Gly Lys Gly Trp Asp
            100                 105                 110

Leu Met Gln Asn Ile Met Asn Asp Met Pro Ile Tyr Met Tyr Ser Val
        115                 120                 125

Cys Asn Val Met Ser Gly Asp Gln Asp Asn Trp Leu Arg Thr Asn Trp
    130                 135                 140

Val Tyr Arg Gly Glu Ala Glu Arg Ile Phe Ile Glu Leu Lys Phe Thr
145                 150                 155                 160

Val Arg Asp Cys Asn Ser Phe Pro Gly Gly Ala Ser Ser Cys Lys Glu
                165                 170                 175

Thr Phe Asn Leu Tyr Tyr Ala Glu Ser Asp Leu Asp Tyr Gly Thr Asn
            180                 185                 190

Phe Gln Lys Arg Leu Phe Thr Lys Ile Asp Thr Ile Ala Pro Asp Glu
        195                 200                 205

Ile Thr Val Ser Ser Asp Phe Glu Ala Arg His Val Lys Leu Asn Val
    210                 215                 220

Glu Glu Arg Ser Val Gly Pro Leu Thr Arg Lys Gly Phe Tyr Leu Ala
225                 230                 235                 240
```

```
Phe Gln Asp Ile Gly Ala Cys Val Ala Leu Leu Ser Val Arg Val Tyr
                245                 250                 255

Tyr Lys Lys Cys Pro Glu Leu Leu Gln Gly Leu Ala His Phe Pro Glu
            260                 265                 270

Thr Ile Ala Gly Ser Asp Ala Pro Ser Leu Ala Thr Val Ala Gly Thr
        275                 280                 285

Cys Val Asp His Ala Val Val Pro Pro Gly Gly Glu Glu Pro Arg Met
    290                 295                 300

His Cys Ala Val Asp Gly Glu Trp Leu Val Pro Ile Gly Gln Cys Leu
305                 310                 315                 320

Cys Gln Ala Gly Tyr Glu Lys Val Glu Asp Ala Cys Gln Ala Cys Ser
                325                 330                 335

Pro Gly Phe Phe Lys Phe Glu Ala Ser Glu Ser Pro Cys Leu Glu Cys
            340                 345                 350

Pro Glu His Thr Leu Pro Ser Pro Glu Gly Ala Thr Ser Cys Glu Cys
        355                 360                 365

Glu Glu Gly Phe Phe Arg Ala Pro Gln Asp Pro Ala Ser Met Pro Cys
    370                 375                 380

Thr Arg Pro Pro Ser Ala Pro His Tyr Leu Thr Ala Val Gly Met Gly
385                 390                 395                 400

Ala Lys Val Glu Leu Arg Trp Thr Pro Pro Gln Asp Ser Gly Gly Arg
                405                 410                 415

Glu Asp Ile Val Tyr Ser Val Thr Cys Glu Gln Cys Trp Pro Glu Ser
            420                 425                 430

Gly Glu Cys Gly Pro Cys Glu Ala Ser Val Arg Tyr Ser Glu Pro Pro
        435                 440                 445

His Gly Leu Thr Arg Thr Ser Val Thr Val Ser Asp Leu Glu Pro His
    450                 455                 460

Met Asn Tyr Thr Phe Thr Val Glu Ala Arg Asn Gly Val Ser Gly Leu
465                 470                 475                 480
```

```
Val Thr Ser Arg Ser Phe Arg Thr Ala Ser Val Ser Ile Asn Gln Thr
                485                 490                 495

Glu Pro Pro Lys Val Arg Leu Glu Gly Arg Ser Thr Thr Ser Leu Ser
            500                 505                 510

Val Ser Trp Ser Ile Pro Pro Pro Gln Gln Ser Arg Val Trp Lys Tyr
        515                 520                 525

Glu Val Thr Tyr Arg Lys Lys Gly Asp Ser Asn Ser Tyr Asn Val Arg
    530                 535                 540

Arg Thr Glu Gly Phe Ser Val Thr Leu Asp Asp Leu Ala Pro Asp Thr
545                 550                 555                 560

Thr Tyr Leu Val Gln Val Gln Ala Leu Thr Gln Glu Gly Gln Gly Ala
                565                 570                 575

Gly Ser Arg Val His Glu Phe Gln Thr Leu Ser Pro Glu Gly Ser Gly
            580                 585                 590

Asn Leu Ala Val Ile Gly Gly Val Ala Val Gly Val Val Leu Leu Leu
        595                 600                 605

Val Leu Ala Gly Val Gly Phe Phe Ile His Arg Arg Arg Lys Asn Gln
    610                 615                 620

Arg Ala Arg Gln Ser Pro Glu Asp Val Tyr Phe Ser Lys Ser Glu Gln
625                 630                 635                 640

Leu Lys Pro Leu Lys Thr Tyr Val Asp Pro His Thr Tyr Glu Asp Pro
                645                 650                 655

Asn Gln Ala Val Leu Lys Phe Thr Thr Glu Ile His Pro Ser Cys Val
            660                 665                 670

Thr Arg Gln Lys Val Ile Gly Ala Gly Glu Phe Gly Glu Val Tyr Lys
        675                 680                 685

Gly Met Leu Lys Thr Ser Ser Gly Lys Lys Glu Val Pro Val Ala Ile
    690                 695                 700

Lys Thr Leu Lys Ala Gly Tyr Thr Glu Lys Gln Arg Val Asp Phe Leu
705                 710                 715                 720
```

```
Gly Glu Ala Gly Ile Met Gly Gln Phe Ser His His Asn Ile Ile Arg
                725                 730                 735

Leu Glu Gly Val Ile Ser Lys Tyr Lys Pro Met Met Ile Ile Thr Glu
            740                 745                 750

Tyr Met Glu Asn Gly Ala Leu Asp Lys Phe Leu Arg Glu Lys Asp Gly
        755                 760                 765

Glu Phe Ser Val Leu Gln Leu Val Gly Met Leu Arg Gly Ile Ala Ala
    770                 775                 780

Gly Met Lys Tyr Leu Ala Asn Met Asn Tyr Val His Arg Asp Leu Ala
785                 790                 795                 800

Ala Arg Asn Ile Leu Val Asn Ser Asn Leu Val Cys Lys Val Ser Asp
                805                 810                 815

Phe Gly Leu Ser Arg Val Leu Glu Asp Asp Pro Glu Ala Thr Tyr Thr
            820                 825                 830

Thr Ser Gly Gly Lys Ile Pro Ile Arg Trp Thr Ala Pro Glu Ala Ile
        835                 840                 845

Ser Tyr Arg Lys Phe Thr Ser Ala Ser Asp Val Trp Ser Phe Gly Ile
    850                 855                 860

Val Met Trp Glu Val Met Thr Tyr Gly Glu Arg Pro Tyr Trp Glu Leu
865                 870                 875                 880

Ser Asn His Glu Val Met Lys Ala Ile Asn Asp Gly Phe Arg Leu Pro
                885                 890                 895

Thr Pro Met Asp Cys Pro Ser Ala Ile Tyr Gln Leu Met Met Gln Cys
            900                 905                 910

Trp Gln Gln Glu Arg Ala Arg Arg Pro Lys Phe Ala Asp Ile Val Ser
        915                 920                 925

Ile Leu Asp Lys Leu Ile Arg Ala Pro Asp Ser Leu Lys Thr Leu Ala
    930                 935                 940

Asp Phe Asp Pro Arg Val Ser Ile Arg Leu Pro Ser Thr Ser Gly Ser
945                 950                 955                 960

Glu Gly Val Pro Phe Arg Thr Val Ser Glu Trp Leu Glu Ser Ile Lys
```

```
                965                 970                 975

Met Gln Gln Tyr Thr Glu His Phe Met Ala Ala Gly Tyr Thr Ala Ile
            980                 985                 990

Glu Lys Val Val Gln Met Thr Asn  Asp Asp Ile Lys Arg  Ile Gly Val
        995                1000                1005

Arg Leu  Pro Gly His Gln Lys  Arg Ile Ala Tyr Ser  Leu Leu Gly
    1010                1015                1020

Leu Lys  Asp Gln Val Asn Thr  Val Gly Ile Pro Ile
    1025                1030                1035
```

<210> 21
<211> 1506
<212> DNA
<213> Homo sapiens

<400> 21

```
cagggcaagg aagtggtact gctggacttt gctgcagctg gaggggagct cggctggctc     60

acacacccgt atggcaaagg gtgggacctg atgcagaaca tcatgaatga catgccgatc    120

tacatgtact ccgtgtgcaa cgtgatgtct ggcgaccagg acaactggct ccgcaccaac    180

tgggtgtacc gaggagaggc tgagcgtatc ttcattgagc tcaagtttac tgtacgtgac    240

tgcaacagct tccctggtgg cgccagctcc tgcaaggaga ctttcaacct ctactatgcc    300

gagtcggacc tggactacgg caccaacttc cagaagcgcc tgttcaccaa gattgacacc    360

attgcgcccg atgagatcac cgtcagcagc gacttcgagg cacgccacgt gaagctgaac    420

gtggaggagc gctccgtggg gccgctcacc cgcaaaggct tctacctggc cttccaggat    480

atcggtgcct gtgtggcgct gctctccgtc cgtgtctact acaagaagtg ccccgagctg    540

ctgcagggcc tggcccactt ccctgagacc atcgccggct ctgatgcacc ttccctggcc    600

actgtggccg gcacctgtgt ggaccatgcc gtggtgccac cgggggtga agagccccgt    660

atgcactgtg cagtggatgg cgagtggctg gtgcccattg ggcagtgcct gtgccaggca    720

ggctacgaga aggtggagga tgcctgccag gcctgctcgc ctggattttt taagtttgag    780

gcatctgaga gcccctgctt ggagtgccct gagcacacgc tgccatcccc tgagggtgcc    840

acctcctgcg agtgtgagga aggcttcttc cgggcacctc aggacccagc gtcgatgcct    900

tgcacacgac ccccctccgc cccacactac ctcacagccg tgggcatggg tgccaaggtg    960

gagctgcgct ggacgccccc tcaggacagc gggggccgcg aggacattgt ctacagcgtc   1020

acctgcgaac agtgctggcc cgagtctggg gaatgcgggc cgtgtgaggc cagtgtgcgc   1080
```

tactcggagc ctcctcacgg actgacccgc accagtgtga cagtgagcga cctggagccc 1140

cacatgaact acaccttcac cgtggaggcc cgcaatggcg tctcaggcct ggtaaccagc 1200

cgcagcttcc gtactgccag tgtcagcatc aaccagacag agccccccaa ggtgaggctg 1260

gagggccgca gcaccacctc gcttagcgtc tcctggagca tccccccgcc gcagcagagc 1320

cgagtgtgga agtacgaggt cacttaccgc aagaagggag actccaacag ctacaatgtg 1380

cgccgcaccg agggtttctc cgtgaccctg gacgacctgg ccccagacac cacctacctg 1440

gtccaggtgc aggcactgac gcaggagggc caggggccg gcagcagggt gcacgaattc 1500

cagacg 1506

<210> 22
<211> 1506
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Human sequence optimized for codon usage in Listeria

<400> 22
caaggtaaag aagttgtttt attagatttt gcagcagcag gtggtgaatt aggttggtta 60

acacatccat atggtaaagg ttgggattta atgcaaaata ttatgaatga tatgccaatt 120

tatatgtata gtgtttgtaa tgttatgagt ggtgatcaag ataattggtt acgtacaaat 180

tgggtttatc gtggtgaagc agaacgtatt tttattgaat taaaatttac agttcgtgat 240

tgtaatagtt ttccaggtgg tgcaagtagt tgtaaagaaa catttaattt atattatgca 300

gaaagtgatt tagattatgg tacaaatttt caaaaacgtt tatttacaaa aattgataca 360

attgcaccag atgaaattac agttagtagt gattttgaag cacgtcatgt taaattaaat 420

gttgaagaac gtagtgttgg tccattaaca cgtaaaggtt tttatttagc atttcaagat 480

attggtgcat gtgttgcatt attaagtgtt cgtgtttatt ataaaaaatg tccagaatta 540

ttacaaggtt tagcacattt tccagaaaca attgcaggta gtgatgcacc aagtttagca 600

acagttgcag gtacatgtgt tgatcatgca gttgttccac caggtggtga agaaccacgt 660

atgcattgtg cagttgatgg tgaatggtta gttccaattg gtcaatgttt atgtcaagca 720

ggttatgaaa aagttgaaga tgcatgtcaa gcatgtagtc caggtttttt taaatttgaa 780

gcaagtgaaa gtccatgttt agaatgtcca gaacatacat taccaagtcc agaaggtgca 840

acaagttgtg aatgtgaaga aggttttttt cgtgcaccac aagatccagc aagtatgcca 900

tgtacacgtc caccaagtgc accacattat ttaacagcag ttggtatggg tgcaaaagtt 960

gaattacgtt ggacaccacc acaagatagt ggtggtcgtg aagatattgt ttatagtgtt 1020

```
acatgtgaac aatgttggcc agaaagtggt gaatgtggtc catgtgaagc aagtgttcgt   1080

tatagtgaac caccacatgg tttaacacgt acaagtgtta cagttagtga tttagaacca   1140

catatgaatt atacatttac agttgaagca cgtaatggtg ttagtggttt agttacaagt   1200

cgtagttttc gtacagcaag tgttagtatt aatcaaacag aaccaccaaa agttcgttta   1260

gaaggtcgta gtacaacaag tttaagtgtt agttggagta ttccaccacc acaacaaagt   1320

cgtgtttgga aatatgaagt tacatatcgt aaaaaaggtg atagtaatag ttataatgtt   1380

cgtcgtacag aaggttttag tgttacatta gatgatttag caccagatac aacatattta   1440

gttcaagttc aagcattaac acaagaaggt caaggtgcag gtagtcgtgt tcatgaattt   1500

caaaca                                                              1506
```

<210> 23
<211> 502
<212> PRT
<213> Homo sapeins

<400> 23

```
Gln Gly Lys Glu Val Val Leu Leu Asp Phe Ala Ala Ala Gly Gly Glu
1               5                   10                  15

Leu Gly Trp Leu Thr His Pro Tyr Gly Lys Gly Trp Asp Leu Met Gln
            20                  25                  30

Asn Ile Met Asn Asp Met Pro Ile Tyr Met Tyr Ser Val Cys Asn Val
        35                  40                  45

Met Ser Gly Asp Gln Asp Asn Trp Leu Arg Thr Asn Trp Val Tyr Arg
    50                  55                  60

Gly Glu Ala Glu Arg Ile Phe Ile Glu Leu Lys Phe Thr Val Arg Asp
65                  70                  75                  80

Cys Asn Ser Phe Pro Gly Gly Ala Ser Ser Cys Lys Glu Thr Phe Asn
                85                  90                  95

Leu Tyr Tyr Ala Glu Ser Asp Leu Asp Tyr Gly Thr Asn Phe Gln Lys
            100                 105                 110

Arg Leu Phe Thr Lys Ile Asp Thr Ile Ala Pro Asp Glu Ile Thr Val
        115                 120                 125

Ser Ser Asp Phe Glu Ala Arg His Val Lys Leu Asn Val Glu Glu Arg
    130                 135                 140
```

```
Ser Val Gly Pro Leu Thr Arg Lys Gly Phe Tyr Leu Ala Phe Gln Asp
145                 150                 155                 160

Ile Gly Ala Cys Val Ala Leu Leu Ser Val Arg Val Tyr Tyr Lys Lys
                165                 170                 175

Cys Pro Glu Leu Leu Gln Gly Leu Ala His Phe Pro Glu Thr Ile Ala
            180                 185                 190

Gly Ser Asp Ala Pro Ser Leu Ala Thr Val Ala Gly Thr Cys Val Asp
        195                 200                 205

His Ala Val Val Pro Pro Gly Gly Glu Glu Pro Arg Met His Cys Ala
    210                 215                 220

Val Asp Gly Glu Trp Leu Val Pro Ile Gly Gln Cys Leu Cys Gln Ala
225                 230                 235                 240

Gly Tyr Glu Lys Val Glu Asp Ala Cys Gln Ala Cys Ser Pro Gly Phe
                245                 250                 255

Phe Lys Phe Glu Ala Ser Glu Ser Pro Cys Leu Glu Cys Pro Glu His
            260                 265                 270

Thr Leu Pro Ser Pro Glu Gly Ala Thr Ser Cys Glu Cys Glu Glu Gly
        275                 280                 285

Phe Phe Arg Ala Pro Gln Asp Pro Ala Ser Met Pro Cys Thr Arg Pro
    290                 295                 300

Pro Ser Ala Pro His Tyr Leu Thr Ala Val Gly Met Gly Ala Lys Val
305                 310                 315                 320

Glu Leu Arg Trp Thr Pro Pro Gln Asp Ser Gly Gly Arg Glu Asp Ile
                325                 330                 335

Val Tyr Ser Val Thr Cys Glu Gln Cys Trp Pro Glu Ser Gly Glu Cys
            340                 345                 350

Gly Pro Cys Glu Ala Ser Val Arg Tyr Ser Glu Pro Pro His Gly Leu
        355                 360                 365

Thr Arg Thr Ser Val Thr Val Ser Asp Leu Glu Pro His Met Asn Tyr
    370                 375                 380
```

```
Thr Phe Thr Val Glu Ala Arg Asn Gly Val Ser Gly Leu Val Thr Ser
385                 390                 395                 400

Arg Ser Phe Arg Thr Ala Ser Val Ser Ile Asn Gln Thr Glu Pro Pro
                405                 410                 415

Lys Val Arg Leu Glu Gly Arg Ser Thr Thr Ser Leu Ser Val Ser Trp
            420                 425                 430

Ser Ile Pro Pro Pro Gln Gln Ser Arg Val Trp Lys Tyr Glu Val Thr
        435                 440                 445

Tyr Arg Lys Lys Gly Asp Ser Asn Ser Tyr Asn Val Arg Arg Thr Glu
    450                 455                 460

Gly Phe Ser Val Thr Leu Asp Asp Leu Ala Pro Asp Thr Thr Tyr Leu
465                 470                 475                 480

Val Gln Val Gln Ala Leu Thr Gln Glu Gly Gln Gly Ala Gly Ser Arg
                485                 490                 495

Val His Glu Phe Gln Thr
            500
```

<210> 24
<211> 1689
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Fusion protein construct

<400> 24

```
atgaaaaaaa taatgctagt ttttattaca cttatattag ttagtctacc aattgcgcaa     60

caaactgaag caaaggatgc atctgcattc aataaagaaa attcaatttc atccatggca    120

ccaccagcat ctccgcctgc aagtcctaag acgccaatcg aaaagaaaca cgcggatcttc    180

gagcagggca aggaagtggt actgctggac tttgctgcag ctggagggga gctcggctgg    240

ctcacacacc cgtatggcaa agggtgggac ctgatgcaga acatcatgaa tgacatgccg    300

atctacatgt actccgtgtg caacgtgatg tctggcgacc aggacaactg gctccgcacc    360

aactgggtgt accgaggaga ggctgagcgt atcttcattg agctcaagtt tactgtacgt    420

gactgcaaca gcttccctgg tggcgccagc tcctgcaagg agactttcaa cctctactat    480

gccgagtcgg acctggacta cggcaccaac ttccagaagc gcctgttcac caagattgac    540

accattgcgc ccgatgagat caccgtcagc agcgacttcg aggcacgcca cgtgaagctg    600
```

aacgtggagg agcgctccgt ggggccgctc acccgcaaag gcttctacct ggccttccag 660

gatatcggtg cctgtgtggc gctgctctcc gtccgtgtct actacaagaa gtgccccgag 720

ctgctgcagg gcctggccca cttccctgag accatcgccg gctctgatgc accttccctg 780

gccactgtgg ccggcacctg tgtggaccat gccgtggtgc caccgggggg tgaagagccc 840

cgtatgcact gtgcagtgga tggcgagtgg ctggtgccca ttgggcagtg cctgtgccag 900

gcaggctacg agaaggtgga ggatgcctgc caggcctgct cgcctggatt ttttaagttt 960

gaggcatctg agagcccctg cttggagtgc cctgagcaca cgctgccatc ccctgagggt 1020

gccacctcct gcgagtgtga ggaaggcttc ttccgggcac ctcaggaccc agcgtcgatg 1080

ccttgcacac gacccccctc cgccccacac tacctcacag ccgtgggcat gggtgccaag 1140

gtggagctgc gctggacgcc ccctcaggac agcgggggcc gcgaggacat tgtctacagc 1200

gtcacctgcg aacagtgctg gcccgagtct ggggaatgcg ggccgtgtga ggccagtgtg 1260

cgctactcgg agcctcctca cggactgacc cgcaccagtg tgacagtgag cgacctggag 1320

ccccacatga actacacctt caccgtggag gcccgcaatg gcgtcttcagg cctggtaacc 1380

agccgcagct tccgtactgc cagtgtcagc atcaaccaga cagagccccc caaggtgagg 1440

ctggagggcc gcagcaccac ctcgcttagc gtctcctgga gcatcccccc gccgcagcag 1500

agccgagtgt ggaagtacga ggtcacttac cgcaagaagg gagactccaa cagctacaat 1560

gtgcgccgca ccgagggttt ctccgtgacc ctggacgacc tggccccaga caccacctac 1620

ctggtccagg tgcaggcact gacgcaggag ggccaggggg ccggcagcag ggtgcacgaa 1680

ttccagacg 1689

<210> 25
<211> 563
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Predicted fusion protein

<400> 25

Met Lys Lys Ile Met Leu Val Phe Ile Thr Leu Ile Leu Val Ser Leu
1 5 10 15

Pro Ile Ala Gln Gln Thr Glu Ala Lys Asp Ala Ser Ala Phe Asn Lys
20 25 30

Glu Asn Ser Ile Ser Ser Met Ala Pro Pro Ala Ser Pro Pro Ala Ser
35 40 45

```
Pro Lys Thr Pro Ile Glu Lys Lys His Ala Asp Leu Glu Gln Gly Lys
    50                  55                  60

Glu Val Val Leu Leu Asp Phe Ala Ala Ala Gly Gly Glu Leu Gly Trp
65                  70                  75                  80

Leu Thr His Pro Tyr Gly Lys Gly Trp Asp Leu Met Gln Asn Ile Met
                85                  90                  95

Asn Asp Met Pro Ile Tyr Met Tyr Ser Val Cys Asn Val Met Ser Gly
            100                 105                 110

Asp Gln Asp Asn Trp Leu Arg Thr Asn Trp Val Tyr Arg Gly Glu Ala
        115                 120                 125

Glu Arg Ile Phe Ile Glu Leu Lys Phe Thr Val Arg Asp Cys Asn Ser
    130                 135                 140

Phe Pro Gly Gly Ala Ser Ser Cys Lys Glu Thr Phe Asn Leu Tyr Tyr
145                 150                 155                 160

Ala Glu Ser Asp Leu Asp Tyr Gly Thr Asn Phe Gln Lys Arg Leu Phe
                165                 170                 175

Thr Lys Ile Asp Thr Ile Ala Pro Asp Glu Ile Thr Val Ser Ser Asp
            180                 185                 190

Phe Glu Ala Arg His Val Lys Leu Asn Val Glu Glu Arg Ser Val Gly
        195                 200                 205

Pro Leu Thr Arg Lys Gly Phe Tyr Leu Ala Phe Gln Asp Ile Gly Ala
    210                 215                 220

Cys Val Ala Leu Leu Ser Val Arg Val Tyr Tyr Lys Lys Cys Pro Glu
225                 230                 235                 240

Leu Leu Gln Gly Leu Ala His Phe Pro Glu Thr Ile Ala Gly Ser Asp
                245                 250                 255

Ala Pro Ser Leu Ala Thr Val Ala Gly Thr Cys Val Asp His Ala Val
            260                 265                 270

Val Pro Pro Gly Gly Glu Glu Pro Arg Met His Cys Ala Val Asp Gly
        275                 280                 285

Glu Trp Leu Val Pro Ile Gly Gln Cys Leu Cys Gln Ala Gly Tyr Glu
```

```
    290                 295                 300

Lys Val Glu Asp Ala Cys Gln Ala Cys Ser Pro Gly Phe Phe Lys Phe
305                 310                 315                 320

Glu Ala Ser Glu Ser Pro Cys Leu Glu Cys Pro Glu His Thr Leu Pro
                325                 330                 335

Ser Pro Glu Gly Ala Thr Ser Cys Glu Cys Glu Glu Gly Phe Phe Arg
            340                 345                 350

Ala Pro Gln Asp Pro Ala Ser Met Pro Cys Thr Arg Pro Pro Ser Ala
        355                 360                 365

Pro His Tyr Leu Thr Ala Val Gly Met Gly Ala Lys Val Glu Leu Arg
    370                 375                 380

Trp Thr Pro Pro Gln Asp Ser Gly Gly Arg Glu Asp Ile Val Tyr Ser
385                 390                 395                 400

Val Thr Cys Glu Gln Cys Trp Pro Glu Ser Gly Glu Cys Gly Pro Cys
                405                 410                 415

Glu Ala Ser Val Arg Tyr Ser Glu Pro Pro His Gly Leu Thr Arg Thr
            420                 425                 430

Ser Val Thr Val Ser Asp Leu Glu Pro His Met Asn Tyr Thr Phe Thr
        435                 440                 445

Val Glu Ala Arg Asn Gly Val Ser Gly Leu Val Thr Ser Arg Ser Phe
    450                 455                 460

Arg Thr Ala Ser Val Ser Ile Asn Gln Thr Glu Pro Pro Lys Val Arg
465                 470                 475                 480

Leu Glu Gly Arg Ser Thr Thr Ser Leu Ser Val Ser Trp Ser Ile Pro
                485                 490                 495

Pro Pro Gln Gln Ser Arg Val Trp Lys Tyr Glu Val Thr Tyr Arg Lys
            500                 505                 510

Lys Gly Asp Ser Asn Ser Tyr Asn Val Arg Arg Thr Glu Gly Phe Ser
        515                 520                 525

Val Thr Leu Asp Asp Leu Ala Pro Asp Thr Thr Tyr Leu Val Gln Val
    530                 535                 540
```

Gln Ala Leu Thr Gln Glu Gly Gln Gly Ala Gly Ser Arg Val His Glu
545 550 555 560

Phe Gln Thr

<210> 26
<211> 1989
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Fusion protein construct

<400> 26
ggtacctcct ttgattagta tattcctatc ttaaagttac ttttatgtgg aggcattaac 60

atttgttaat gacgtcaaaa ggatagcaag actagaataa agctataaag caagcatata 120

atattgcgtt tcatctttag aagcgaattt cgccaatatt ataattatca aaagagaggg 180

gtggcaaacg gtatttggca ttattaggtt aaaaaatgta gaaggagagt gaaacccatg 240

aaaaaaataa tgctagtttt tattacactt atattagtta gtctaccaat tgcgcaacaa 300

actgaagcaa aggatgcatc tgcattcaat aaagaaaatt caatttcatc catggcacca 360

ccagcatctc cgcctgcaag tcctaagacg ccaatcgaaa agaaacacgc ggatggatcc 420

gattataaag atgatgatga taaacaaggt aaagaagttg ttttattaga ttttgcagca 480

gcaggtggtg aattaggttg gttaacacat ccatatggta aaggttggga tttaatgcaa 540

aatattatga atgatatgcc aatttatatg tatagtgttt gtaatgttat gagtggtgat 600

caagataatt ggttacgtac aaattgggtt tatcgtggtg aagcagaacg tatttttatt 660

gaattaaaat ttacagttcg tgattgtaat agttttccag gtggtgcaag tagttgtaaa 720

gaaacattta atttatatta tgcagaaagt gatttagatt atggtacaaa ttttcaaaaa 780

cgtttattta caaaaattga tacaattgca ccagatgaaa ttacagttag tagtgatttt 840

gaagcacgtc atgttaaatt aaatgttgaa gaacgtagtg ttggtccatt aacacgtaaa 900

ggtttttatt tagcatttca agatattggt gcatgtgttg cattattaag tgttcgtgtt 960

tattataaaa aatgtccaga attattacaa ggtttagcac attttccaga aacaattgca 1020

ggtagtgatg caccaagttt agcaacagtt gcaggtacat gtgttgatca tgcagttgtt 1080

ccaccaggtg gtgaagaacc acgtatgcat tgtgcagttg atggtgaatg gttagttcca 1140

attggtcaat gtttatgtca agcaggttat gaaaaagttg aagatgcatg tcaagcatgt 1200

agtccaggtt tttttaaatt tgaagcaagt gaaagtccat gtttagaatg tccagaacat 1260

acattaccaa gtccagaagg tgcaacaagt tgtgaatgtg aagaaggttt ttttcgtgca 1320

ccacaagatc cagcaagtat gccatgtaca cgtccaccaa gtgcaccaca ttatttaaca 1380

gcagttggta tgggtgcaaa agttgaatta cgttggacac caccacaaga tagtggtggt 1440

cgtgaagata ttgtttatag tgttacatgt gaacaatgtt ggccagaaag tggtgaatgt 1500

ggtccatgtg aagcaagtgt tcgttatagt gaaccaccac atggtttaac acgtacaagt 1560

gttacagtta gtgatttaga accacatatg aattatacat ttacagttga agcacgtaat 1620

ggtgttagtg gtttagttac aagtcgtagt tttcgtacag caagtgttag tattaatcaa 1680

acagaaccac caaaagttcg tttagaaggt cgtagtacaa caagtttaag tgttagttgg 1740

agtattccac caccacaaca aagtcgtgtt tggaaatatg aagttacata tcgtaaaaaa 1800

ggtgatagta atagttataa tgttcgtcgt acagaaggtt ttagtgttac attagatgat 1860

ttagcaccag atacaacata tttagttcaa gttcaagcat taacacaaga aggtcaaggt 1920

gcaggtagtc gtgttcatga atttcaaaca gaacaaaaat taattagtga agaagattta 1980

tgagagctc 1989

<210> 27
<211> 581
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Predicted fusion protein

<400> 27

Met Lys Lys Ile Met Leu Val Phe Ile Thr Leu Ile Leu Val Ser Leu
1 5 10 15

Pro Ile Ala Gln Gln Thr Glu Ala Lys Asp Ala Ser Ala Phe Asn Lys
20 25 30

Glu Asn Ser Ile Ser Ser Met Ala Pro Pro Ala Ser Pro Pro Ala Ser
35 40 45

Pro Lys Thr Pro Ile Glu Lys Lys His Ala Asp Gly Ser Asp Tyr Lys
50 55 60

Asp Asp Asp Asp Lys Gln Gly Lys Glu Val Val Leu Leu Asp Phe Ala
65 70 75 80

Ala Ala Gly Gly Glu Leu Gly Trp Leu Thr His Pro Tyr Gly Lys Gly
85 90 95

```
Trp Asp Leu Met Gln Asn Ile Met Asn Asp Met Pro Ile Tyr Met Tyr
            100                 105                 110

Ser Val Cys Asn Val Met Ser Gly Asp Gln Asp Asn Trp Leu Arg Thr
        115                 120                 125

Asn Trp Val Tyr Arg Gly Glu Ala Glu Arg Ile Phe Ile Glu Leu Lys
    130                 135                 140

Phe Thr Val Arg Asp Cys Asn Ser Phe Pro Gly Gly Ala Ser Ser Cys
145                 150                 155                 160

Lys Glu Thr Phe Asn Leu Tyr Tyr Ala Glu Ser Asp Leu Asp Tyr Gly
                165                 170                 175

Thr Asn Phe Gln Lys Arg Leu Phe Thr Lys Ile Asp Thr Ile Ala Pro
            180                 185                 190

Asp Glu Ile Thr Val Ser Ser Asp Phe Glu Ala Arg His Val Lys Leu
        195                 200                 205

Asn Val Glu Glu Arg Ser Val Gly Pro Leu Thr Arg Lys Gly Phe Tyr
    210                 215                 220

Leu Ala Phe Gln Asp Ile Gly Ala Cys Val Ala Leu Leu Ser Val Arg
225                 230                 235                 240

Val Tyr Tyr Lys Lys Cys Pro Glu Leu Leu Gln Gly Leu Ala His Phe
                245                 250                 255

Pro Glu Thr Ile Ala Gly Ser Asp Ala Pro Ser Leu Ala Thr Val Ala
            260                 265                 270

Gly Thr Cys Val Asp His Ala Val Val Pro Pro Gly Gly Glu Glu Pro
        275                 280                 285

Arg Met His Cys Ala Val Asp Gly Glu Trp Leu Val Pro Ile Gly Gln
    290                 295                 300

Cys Leu Cys Gln Ala Gly Tyr Glu Lys Val Glu Asp Ala Cys Gln Ala
305                 310                 315                 320

Cys Ser Pro Gly Phe Phe Lys Phe Glu Ala Ser Glu Ser Pro Cys Leu
                325                 330                 335

Glu Cys Pro Glu His Thr Leu Pro Ser Pro Glu Gly Ala Thr Ser Cys
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            340                 345                 350

Glu Cys Glu Glu Gly Phe Phe Arg Ala Pro Gln Asp Pro Ala Ser Met
        355                 360                 365

Pro Cys Thr Arg Pro Pro Ser Ala Pro His Tyr Leu Thr Ala Val Gly
    370                 375                 380

Met Gly Ala Lys Val Glu Leu Arg Trp Thr Pro Pro Gln Asp Ser Gly
385                 390                 395                 400

Gly Arg Glu Asp Ile Val Tyr Ser Val Thr Cys Glu Gln Cys Trp Pro
                405                 410                 415

Glu Ser Gly Glu Cys Gly Pro Cys Glu Ala Ser Val Arg Tyr Ser Glu
            420                 425                 430

Pro Pro His Gly Leu Thr Arg Thr Ser Val Thr Val Ser Asp Leu Glu
        435                 440                 445

Pro His Met Asn Tyr Thr Phe Thr Val Glu Ala Arg Asn Gly Val Ser
    450                 455                 460

Gly Leu Val Thr Ser Arg Ser Phe Arg Thr Ala Ser Val Ser Ile Asn
465                 470                 475                 480

Gln Thr Glu Pro Pro Lys Val Arg Leu Glu Gly Arg Ser Thr Thr Ser
                485                 490                 495

Leu Ser Val Ser Trp Ser Ile Pro Pro Pro Gln Gln Ser Arg Val Trp
            500                 505                 510

Lys Tyr Glu Val Thr Tyr Arg Lys Lys Gly Asp Ser Asn Ser Tyr Asn
        515                 520                 525

Val Arg Arg Thr Glu Gly Phe Ser Val Thr Leu Asp Asp Leu Ala Pro
    530                 535                 540

Asp Thr Thr Tyr Leu Val Gln Val Gln Ala Leu Thr Gln Glu Gly Gln
545                 550                 555                 560

Gly Ala Gly Ser Arg Val His Glu Phe Gln Thr Glu Gln Lys Leu Ile
                565                 570                 575

Ser Glu Glu Asp Leu
            580
```

<210> 28
<211> 1989
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Construct for fusion protein

<400> 28
ggtacctcct ttgattagta tattcctatc ttaaagttac ttttatgtgg aggcattaac 60

atttgttaat gacgtcaaaa ggatagcaag actagaataa agctataaag caagcatata 120

atattgcgtt tcatctttag aagcgaattt cgccaatatt ataattatca aaagagaggg 180

gtggcaaacg gtatttggca ttattaggtt aaaaaatgta gaaggagagt gaaacccatg 240

aaaaaaatta tgttagtttt tattacatta attttagtta gtttaccaat tgcacaacaa 300

acagaagcaa aagatgcaag tgcatttaat aaagaaaata gtattagtag tatggcacca 360

ccagcaagtc caccagcaag tccaaaaaca ccaattgaaa aaaaacatgc agatggatcc 420

gattataaag atgatgatga taaacaaggt aaagaagttg ttttattaga ttttgcagca 480

gcaggtggtg aattaggttg gttaacacat ccatatggta aaggttggga tttaatgcaa 540

aatattatga atgatatgcc aatttatatg tatagtgttt gtaatgttat gagtggtgat 600

caagataatt ggttacgtac aaattgggtt tatcgtggtg aagcagaacg tatttttatt 660

gaattaaaat ttacagttcg tgattgtaat agttttccag gtggtgcaag tagttgtaaa 720

gaaacattta atttatatta tgcagaaagt gatttagatt atggtacaaa ttttcaaaaa 780

cgtttattta caaaaattga tacaattgca ccagatgaaa ttacagttag tagtgatttt 840

gaagcacgtc atgttaaatt aaatgttgaa gaacgtagtg ttggtccatt aacacgtaaa 900

ggtttttatt tagcatttca agatattggt gcatgtgttg cattattaag tgttcgtgtt 960

tattataaaa aatgtccaga attattacaa ggtttagcac attttccaga aacaattgca 1020

ggtagtgatg caccaagttt agcaacagtt gcaggtacat gtgttgatca tgcagttgtt 1080

ccaccaggtg gtgaagaacc acgtatgcat tgtgcagttg atggtgaatg gttagttcca 1140

attggtcaat gtttatgtca agcaggttat gaaaaagttg aagatgcatg tcaagcatgt 1200

agtccaggtt tttttaaatt tgaagcaagt gaaagtccat gtttagaatg tccagaacat 1260

acattaccaa gtccagaagg tgcaacaagt tgtgaatgtg aagaaggttt ttttcgtgca 1320

ccacaagatc cagcaagtat gccatgtaca cgtccaccaa gtgcaccaca ttatttaaca 1380

gcagttggta tgggtgcaaa agttgaatta cgttggacac caccacaaga tagtggtggt 1440

cgtgaagata ttgtttatag tgttacatgt gaacaatgtt ggccagaaag tggtgaatgt 1500

ggtccatgtg aagcaagtgt tcgttatagt gaaccaccac atggtttaac acgtacaagt 1560

gttacagtta gtgatttaga accacatatg aattatacat ttacagttga agcacgtaat 1620

ggtgttagtg gtttagttac aagtcgtagt tttcgtacag caagtgttag tattaatcaa 1680

acagaaccac caaaagttcg tttagaaggt cgtagtacaa caagtttaag tgttagttgg 1740

agtattccac caccacaaca aagtcgtgtt tggaaatatg aagttacata tcgtaaaaaa 1800

ggtgatagta atagttataa tgttcgtcgt acagaaggtt ttagtgttac attagatgat 1860

ttagcaccag atacaacata tttagttcaa gttcaagcat taacacaaga aggtcaaggt 1920

gcaggtagtc gtgttcatga atttcaaaca gaacaaaaat taattagtga agaagattta 1980

tgagagctc 1989

<210> 29
<211> 581
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Predicted Fusion protein

<400> 29

Met Lys Lys Ile Met Leu Val Phe Ile Thr Leu Ile Leu Val Ser Leu
1 5 10 15

Pro Ile Ala Gln Gln Thr Glu Ala Lys Asp Ala Ser Ala Phe Asn Lys
20 25 30

Glu Asn Ser Ile Ser Ser Met Ala Pro Pro Ala Ser Pro Pro Ala Ser
35 40 45

Pro Lys Thr Pro Ile Glu Lys Lys His Ala Asp Gly Ser Asp Tyr Lys
50 55 60

Asp Asp Asp Asp Lys Gln Gly Lys Glu Val Val Leu Leu Asp Phe Ala
65 70 75 80

Ala Ala Gly Gly Glu Leu Gly Trp Leu Thr His Pro Tyr Gly Lys Gly
85 90 95

Trp Asp Leu Met Gln Asn Ile Met Asn Asp Met Pro Ile Tyr Met Tyr
100 105 110

Ser Val Cys Asn Val Met Ser Gly Asp Gln Asp Asn Trp Leu Arg Thr
115 120 125

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Asn Trp Val Tyr Arg Gly Glu Ala Glu Arg Ile Phe Ile Glu Leu Lys
    130                 135                 140

Phe Thr Val Arg Asp Cys Asn Ser Phe Pro Gly Gly Ala Ser Ser Cys
145                 150                 155                 160

Lys Glu Thr Phe Asn Leu Tyr Tyr Ala Glu Ser Asp Leu Asp Tyr Gly
                165                 170                 175

Thr Asn Phe Gln Lys Arg Leu Phe Thr Lys Ile Asp Thr Ile Ala Pro
            180                 185                 190

Asp Glu Ile Thr Val Ser Ser Asp Phe Glu Ala Arg His Val Lys Leu
        195                 200                 205

Asn Val Glu Glu Arg Ser Val Gly Pro Leu Thr Arg Lys Gly Phe Tyr
    210                 215                 220

Leu Ala Phe Gln Asp Ile Gly Ala Cys Val Ala Leu Leu Ser Val Arg
225                 230                 235                 240

Val Tyr Tyr Lys Lys Cys Pro Glu Leu Leu Gln Gly Leu Ala His Phe
                245                 250                 255

Pro Glu Thr Ile Ala Gly Ser Asp Ala Pro Ser Leu Ala Thr Val Ala
            260                 265                 270

Gly Thr Cys Val Asp His Ala Val Val Pro Pro Gly Gly Glu Glu Pro
        275                 280                 285

Arg Met His Cys Ala Val Asp Gly Glu Trp Leu Val Pro Ile Gly Gln
    290                 295                 300

Cys Leu Cys Gln Ala Gly Tyr Glu Lys Val Glu Asp Ala Cys Gln Ala
305                 310                 315                 320

Cys Ser Pro Gly Phe Phe Lys Phe Glu Ala Ser Glu Ser Pro Cys Leu
                325                 330                 335

Glu Cys Pro Glu His Thr Leu Pro Ser Pro Glu Gly Ala Thr Ser Cys
            340                 345                 350

Glu Cys Glu Glu Gly Phe Phe Arg Ala Pro Gln Asp Pro Ala Ser Met
        355                 360                 365

Pro Cys Thr Arg Pro Pro Ser Ala Pro His Tyr Leu Thr Ala Val Gly
```

370 375 380

Met Gly Ala Lys Val Glu Leu Arg Trp Thr Pro Pro Gln Asp Ser Gly
385 390 395 400

Gly Arg Glu Asp Ile Val Tyr Ser Val Thr Cys Glu Gln Cys Trp Pro
405 410 415

Glu Ser Gly Glu Cys Gly Pro Cys Glu Ala Ser Val Arg Tyr Ser Glu
420 425 430

Pro Pro His Gly Leu Thr Arg Thr Ser Val Thr Val Ser Asp Leu Glu
435 440 445

Pro His Met Asn Tyr Thr Phe Thr Val Glu Ala Arg Asn Gly Val Ser
450 455 460

Gly Leu Val Thr Ser Arg Ser Phe Arg Thr Ala Ser Val Ser Ile Asn
465 470 475 480

Gln Thr Glu Pro Pro Lys Val Arg Leu Glu Gly Arg Ser Thr Thr Ser
485 490 495

Leu Ser Val Ser Trp Ser Ile Pro Pro Pro Gln Gln Ser Arg Val Trp
500 505 510

Lys Tyr Glu Val Thr Tyr Arg Lys Lys Gly Asp Ser Asn Ser Tyr Asn
515 520 525

Val Arg Arg Thr Glu Gly Phe Ser Val Thr Leu Asp Asp Leu Ala Pro
530 535 540

Asp Thr Thr Tyr Leu Val Gln Val Gln Ala Leu Thr Gln Glu Gly Gln
545 550 555 560

Gly Ala Gly Ser Arg Val His Glu Phe Gln Thr Glu Gln Lys Leu Ile
565 570 575

Ser Glu Glu Asp Leu
580

<210> 30
<211> 1968
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Fusion protein construct

<400> 30

ggtacctcct ttgattagta tattcctatc ttaaagttac ttttatgtgg aggcattaac 60

atttgttaat gacgtcaaaa ggatagcaag actagaataa agctataaag caagcatata 120

atattgcgtt tcatctttag aagcgaattt cgccaatatt ataattatca aaagagaggg 180

gtggcaaacg gtatttggca ttattaggtt aaaaaatgta gaaggagagt gaaacccatg 240

gcatacgaca gtcgttttga tgaatgggta cagaaactga aagaggaaag ctttcaaaac 300

aatacgtttg accgccgcaa atttattcaa ggagcgggga agattgcagg actttctctt 360

ggattaacga ttgcccagtc ggttggggcc tttggatccg attataaaga tgatgatgat 420

aaacaaggta aagaagttgt tttattagat tttgcagcag caggtggtga attaggttgg 480

ttaacacatc catatggtaa aggttgggat ttaatgcaaa atattatgaa tgatatgcca 540

atttatatgt atagtgtttg taatgttatg agtggtgatc aagataattg gttacgtaca 600

aattgggttt atcgtggtga agcagaacgt atttttattg aattaaaatt tacagttcgt 660

gattgtaata gttttccagg tggtgcaagt agttgtaaag aaacatttaa tttatattat 720

gcagaaagtg atttagatta tggtacaaat tttcaaaaac gtttatttac aaaaattgat 780

acaattgcac cagatgaaat tacagttagt agtgattttg aagcacgtca tgttaaatta 840

aatgttgaag aacgtagtgt tggtccatta acacgtaaag gtttttattt agcatttcaa 900

gatattggtg catgtgttgc attattaagt gttcgtgttt attataaaaa atgtccagaa 960

ttattacaag gtttagcaca ttttccagaa acaattgcag gtagtgatgc accaagttta 1020

gcaacagttg caggtacatg tgttgatcat gcagttgttc caccaggtgg tgaagaacca 1080

cgtatgcatt gtgcagttga tggtgaatgg ttagttccaa ttggtcaatg tttatgtcaa 1140

gcaggttatg aaaaagttga agatgcatgt caagcatgta gtccaggttt ttttaaattt 1200

gaagcaagtg aaagtccatg tttagaatgt ccagaacata cattaccaag tccagaaggt 1260

gcaacaagtt gtgaatgtga agaaggtttt tttcgtgcac cacaagatcc agcaagtatg 1320

ccatgtacac gtccaccaag tgcaccacat tatttaacag cagttggtat gggtgcaaaa 1380

gttgaattac gttggacacc accacaagat agtggtggtc gtgaagatat tgtttatagt 1440

gttacatgtg aacaatgttg gccagaaagt ggtgaatgtg gtccatgtga agcaagtgtt 1500

cgttatagtg aaccaccaca tggtttaaca cgtacaagtg ttacagttag tgatttagaa 1560

ccacatatga attatacatt tacagttgaa gcacgtaatg gtgttagtgg tttagttaca 1620

agtcgtagtt ttcgtacagc aagtgttagt attaatcaaa cagaaccacc aaaagttcgt 1680

ttagaaggtc gtagtacaac aagtttaagt gttagttgga gtattccacc accacaacaa 1740

agtcgtgttt ggaaatatga agttacatat cgtaaaaaag gtgatagtaa tagttataat 1800

gttcgtcgta cagaaggttt tagtgttaca ttagatgatt tagcaccaga tacaacatat 1860

ttagttcaag ttcaagcatt aacacaagaa ggtcaaggtg caggtagtcg tgttcatgaa 1920

tttcaaacag aacaaaaatt aattagtgaa gaagatttat gagagctc 1968

<210> 31
<211> 574
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Predicted Fusion Protein

<400> 31

Met Ala Tyr Asp Ser Arg Phe Asp Glu Trp Val Gln Lys Leu Lys Glu
1 5 10 15

Glu Ser Phe Gln Asn Asn Thr Phe Asp Arg Arg Lys Phe Ile Gln Gly
20 25 30

Ala Gly Lys Ile Ala Gly Leu Ser Leu Gly Leu Thr Ile Ala Gln Ser
35 40 45

Val Gly Ala Phe Gly Ser Asp Tyr Lys Asp Asp Asp Asp Lys Gln Gly
50 55 60

Lys Glu Val Val Leu Leu Asp Phe Ala Ala Ala Gly Gly Glu Leu Gly
65 70 75 80

Trp Leu Thr His Pro Tyr Gly Lys Gly Trp Asp Leu Met Gln Asn Ile
85 90 95

Met Asn Asp Met Pro Ile Tyr Met Tyr Ser Val Cys Asn Val Met Ser
100 105 110

Gly Asp Gln Asp Asn Trp Leu Arg Thr Asn Trp Val Tyr Arg Gly Glu
115 120 125

Ala Glu Arg Ile Phe Ile Glu Leu Lys Phe Thr Val Arg Asp Cys Asn
130 135 140

Ser Phe Pro Gly Gly Ala Ser Ser Cys Lys Glu Thr Phe Asn Leu Tyr
145 150 155 160

Tyr Ala Glu Ser Asp Leu Asp Tyr Gly Thr Asn Phe Gln Lys Arg Leu
165 170 175

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Phe Thr Lys Ile Asp Thr Ile Ala Pro Asp Glu Ile Thr Val Ser Ser
            180                 185                 190

Asp Phe Glu Ala Arg His Val Lys Leu Asn Val Glu Glu Arg Ser Val
        195                 200                 205

Gly Pro Leu Thr Arg Lys Gly Phe Tyr Leu Ala Phe Gln Asp Ile Gly
    210                 215                 220

Ala Cys Val Ala Leu Leu Ser Val Arg Val Tyr Tyr Lys Lys Cys Pro
225                 230                 235                 240

Glu Leu Leu Gln Gly Leu Ala His Phe Pro Glu Thr Ile Ala Gly Ser
                245                 250                 255

Asp Ala Pro Ser Leu Ala Thr Val Ala Gly Thr Cys Val Asp His Ala
            260                 265                 270

Val Val Pro Pro Gly Gly Glu Glu Pro Arg Met His Cys Ala Val Asp
        275                 280                 285

Gly Glu Trp Leu Val Pro Ile Gly Gln Cys Leu Cys Gln Ala Gly Tyr
    290                 295                 300

Glu Lys Val Glu Asp Ala Cys Gln Ala Cys Ser Pro Gly Phe Phe Lys
305                 310                 315                 320

Phe Glu Ala Ser Glu Ser Pro Cys Leu Glu Cys Pro Glu His Thr Leu
                325                 330                 335

Pro Ser Pro Glu Gly Ala Thr Ser Cys Glu Cys Glu Glu Gly Phe Phe
            340                 345                 350

Arg Ala Pro Gln Asp Pro Ala Ser Met Pro Cys Thr Arg Pro Pro Ser
        355                 360                 365

Ala Pro His Tyr Leu Thr Ala Val Gly Met Gly Ala Lys Val Glu Leu
    370                 375                 380

Arg Trp Thr Pro Pro Gln Asp Ser Gly Gly Arg Glu Asp Ile Val Tyr
385                 390                 395                 400

Ser Val Thr Cys Glu Gln Cys Trp Pro Glu Ser Gly Glu Cys Gly Pro
                405                 410                 415
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Cys Glu Ala Ser Val Arg Tyr Ser Glu Pro Pro His Gly Leu Thr Arg
            420                 425                 430

Thr Ser Val Thr Val Ser Asp Leu Glu Pro His Met Asn Tyr Thr Phe
        435                 440                 445

Thr Val Glu Ala Arg Asn Gly Val Ser Gly Leu Val Thr Ser Arg Ser
    450                 455                 460

Phe Arg Thr Ala Ser Val Ser Ile Asn Gln Thr Glu Pro Pro Lys Val
465                 470                 475                 480

Arg Leu Glu Gly Arg Ser Thr Thr Ser Leu Ser Val Ser Trp Ser Ile
                485                 490                 495

Pro Pro Pro Gln Gln Ser Arg Val Trp Lys Tyr Glu Val Thr Tyr Arg
            500                 505                 510

Lys Lys Gly Asp Ser Asn Ser Tyr Asn Val Arg Arg Thr Glu Gly Phe
        515                 520                 525

Ser Val Thr Leu Asp Asp Leu Ala Pro Asp Thr Thr Tyr Leu Val Gln
    530                 535                 540

Val Gln Ala Leu Thr Gln Glu Gly Gln Gly Ala Gly Ser Arg Val His
545                 550                 555                 560

Glu Phe Gln Thr Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu
                565                 570
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<210> 32
<211> 1254
<212> DNA
<213> Homo sapiens

<400> 32
caccgcagga ggaagaacca gcgtgcccgc cagtccccgg aggacgttta cttctccaag 60

tcagaacaac tgaagcccct gaagacatac gtggaccccc acacatatga ggaccccaac 120

caggctgtgt tgaagttcac taccgagatc catccatcct gtgtcactcg gcagaaggtg 180

atcggagcag gagagtttgg ggaggtgtac aagggcatgc tgaagacatc ctcggggaag 240

aaggaggtgc cggtggccat caagacgctg aaagccggct acacagagaa gcagcgagtg 300

gacttcctcg gcgaggccgg catcatgggc cagttcagcc accacaacat catccgccta 360

gagggcgtca tctccaaata caagcccatg atgatcatca ctgagtacat ggagaatggg 420

gccctggaca agttccttcg ggagaaggat ggcgagttca gcgtgctgca gctggtgggc 480

atgctgcggg gcatcgcagc tggcatgaag tacctggcca acatgaacta tgtgcaccgt 540

gacctggctg cccgcaacat cctcgtcaac agcaacctgg tctgcaaggt gtctgacttt 600

ggcctgtccc gcgtgctgga ggacgacccc gaggccacct acaccaccag tggcggcaag 660

atccccatcc gctggaccgc cccggaggcc atttcctacc ggaagttcac ctctgccagc 720

gacgtgtgga gctttggcat tgtcatgtgg gaggtgatga cctatggcga gcggccctac 780

tgggagttgt ccaaccacga ggtgatgaaa gccatcaatg atggcttccg gctccccaca 840

cccatggact gcccctccgc catctaccag ctcatgatgc agtgctggca gcaggagcgt 900

gcccgccgcc ccaagttcgc tgacatcgtc agcatcctgg acaagctcat tcgtgcccct 960

gactccctca agaccctggc tgactttgac ccccgcgtgt ctatccggct ccccagcacg 1020

agcggctcgg agggggtgcc cttccgcacg gtgtccgagt ggctggagtc catcaagatg 1080

cagcagtata cggagcactt catggcggcc ggctacactg ccatcgagaa ggtggtgcag 1140

atgaccaacg acgacatcaa gaggattggg gtgcggctgc ccggccacca gaagcgcatc 1200

gcctacagcc tgctgggact caaggaccag gtgaacactg tggggatccc catc 1254

<210> 33
<211> 1254
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Sequence Optimized for codon usage in Listeria

<400> 33
cacagacgta gaaaaaatca acgtgctcga caatccccag aagatgtgta tttttcgaaa 60

agtgaacaat taaaaccatt aaaaacttat gttgatccgc atacgtacga agacccaaat 120

caagcagtat taaaatttac aacagaaata cacccaagtt gtgttacaag acaaaaagtt 180

attggagcag gtgaattcgg agaggtatat aaaggtatgt taaaaacatc atcaggtaaa 240

aaagaagttc cggttgcaat taaaacctta aaggcaggat atacagaaaa acagcgagtt 300

gatttttag gtgaagcagg aattatgggt caatttagcc atcataatat tattcgtttg 360

gaaggagtaa taagtaaata taaaccaatg atgattatta cagaatacat ggaaaacggt 420

gctttagata aatttttacg tgaaaaggat ggtgaattta gtgttttaca attggttggt 480

atgttaagag gaattgctgc aggtatgaaa tatttagcta atatgaatta tgttcaccgt 540

gatttggcag caagaaatat cctagtcaat tccaatttag tatgtaaagt tagtgatttt 600

ggtttaagca gagtattaga agacgatcca gaggcaacct atacaacatc gggaggtaaa 660

attcctattc gttggacagc accagaagct atcagttacc gtaaatttac aagtgcatca 720

gacgtgtgga gttttgggat tgtaatgtgg gaagttatga catatggaga aagaccatat 780

tgggaattaa gtaatcatga agttatgaaa gcaattaacg atggatttag attaccaact 840

ccgatggatt gtccatctgc catttatcaa ctaatgatgc aatgttggca acaagaaaga 900

gcacgacgtc caaaatttgc agatattgtt agtattttag acaaattaat tcgtgcacca 960

gatagtttaa aaactttagc agactttgat cctcgtgtta gtattcgatt accaagtacg 1020

tcaggttccg aaggagttcc atttcgcaca gtctccgaat ggttggaatc aattaaaatg 1080

caacaataca ccgaacactt tatggcagca ggttacacag caatcgaaaa agttgttcaa 1140

atgacaaatg atgatattaa acgtattgga gttagattac caggccacca gaaacgtatt 1200

gcatattctt tattaggttt aaaagatcaa gttaataccg tgggaattcc aatt 1254

<210> 34
<211> 456
<212> PRT
<213> Homo sapiens

<400> 34

Val His Glu Phe Gln Thr Leu Ser Pro Glu Gly Ser Gly Asn Leu Ala
1 5 10 15

Val Ile Gly Gly Val Ala Val Gly Val Val Leu Leu Leu Val Leu Ala
20 25 30

Gly Val Gly Phe Phe Ile His Arg Arg Arg Lys Asn Gln Arg Ala Arg
35 40 45

Gln Ser Pro Glu Asp Val Tyr Phe Ser Lys Ser Glu Gln Leu Lys Pro
50 55 60

Leu Lys Thr Tyr Val Asp Pro His Thr Tyr Glu Asp Pro Asn Gln Ala
65 70 75 80

Val Leu Lys Phe Thr Thr Glu Ile His Pro Ser Cys Val Thr Arg Gln
85 90 95

Lys Val Ile Gly Ala Gly Glu Phe Gly Glu Val Tyr Lys Gly Met Leu
100 105 110

Lys Thr Ser Ser Gly Lys Lys Glu Val Pro Val Ala Ile Lys Thr Leu
115 120 125

Lys Ala Gly Tyr Thr Glu Lys Gln Arg Val Asp Phe Leu Gly Glu Ala

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    130                 135                 140

Gly Ile Met Gly Gln Phe Ser His His Asn Ile Ile Arg Leu Glu Gly
145                 150                 155                 160

Val Ile Ser Lys Tyr Lys Pro Met Met Ile Ile Thr Glu Tyr Met Glu
                165                 170                 175

Asn Gly Ala Leu Asp Lys Phe Leu Arg Glu Lys Asp Gly Glu Phe Ser
            180                 185                 190

Val Leu Gln Leu Val Gly Met Leu Arg Gly Ile Ala Ala Gly Met Lys
        195                 200                 205

Tyr Leu Ala Asn Met Asn Tyr Val His Arg Asp Leu Ala Ala Arg Asn
    210                 215                 220

Ile Leu Val Asn Ser Asn Leu Val Cys Lys Val Ser Asp Phe Gly Leu
225                 230                 235                 240

Ser Arg Val Leu Glu Asp Asp Pro Glu Ala Thr Tyr Thr Thr Ser Gly
                245                 250                 255

Gly Lys Ile Pro Ile Arg Trp Thr Ala Pro Glu Ala Ile Ser Tyr Arg
            260                 265                 270

Lys Phe Thr Ser Ala Ser Asp Val Trp Ser Phe Gly Ile Val Met Trp
        275                 280                 285

Glu Val Met Thr Tyr Gly Glu Arg Pro Tyr Trp Glu Leu Ser Asn His
    290                 295                 300

Glu Val Met Lys Ala Ile Asn Asp Gly Phe Arg Leu Pro Thr Pro Met
305                 310                 315                 320

Asp Cys Pro Ser Ala Ile Tyr Gln Leu Met Met Gln Cys Trp Gln Gln
                325                 330                 335

Glu Arg Ala Arg Arg Pro Lys Phe Ala Asp Ile Val Ser Ile Leu Asp
            340                 345                 350

Lys Leu Ile Arg Ala Pro Asp Ser Leu Lys Thr Leu Ala Asp Phe Asp
        355                 360                 365

Pro Arg Val Ser Ile Arg Leu Pro Ser Thr Ser Gly Ser Glu Gly Val
    370                 375                 380
```

```
Pro Phe Arg Thr Val Ser Glu Trp Leu Glu Ser Ile Lys Met Gln Gln
385                 390                 395                 400

Tyr Thr Glu His Phe Met Ala Ala Gly Tyr Thr Ala Ile Glu Lys Val
                405                 410                 415

Val Gln Met Thr Asn Asp Asp Ile Lys Arg Ile Gly Val Arg Leu Pro
            420                 425                 430

Gly His Gln Lys Arg Ile Ala Tyr Ser Leu Leu Gly Leu Lys Asp Gln
        435                 440                 445

Val Asn Thr Val Gly Ile Pro Ile
    450                 455
```

<210> 35
<211> 1437
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Fusion Protein

<400> 35

```
atgaaaaaaa taatgctagt ttttattaca cttatattag ttagtctacc aattgcgcaa     60

caaactgaag caaaggatgc atctgcattc aataaagaaa attcaatttc atccatggca    120

ccaccagcat ctccgcctgc aagtcctaag acgccaatcg aaaagaaaca cgcggatctc    180

gagcaccgca ggaggaagaa ccagcgtgcc cgccagtccc cggaggacgt ttacttctcc    240

aagtcagaac aactgaagcc cctgaagaca tacgtggacc cccacacata tgaggacccc    300

aaccaggctg tgttgaagtt cactaccgag atccatccat cctgtgtcac tcggcagaag    360

gtgatcggag caggagagtt tggggaggtg tacaagggca tgctgaagac atcctcgggg    420

aagaaggagg tgccggtggc catcaagacg ctgaaagccg gctacacaga gaagcagcga    480

gtggacttcc tcggcgaggc cggcatcatg ggccagttca gccaccacaa catcatccgc    540

ctagagggcg tcatctccaa atacaagccc atgatgatca tcactgagta catggagaat    600

ggggccctgg acaagttcct tcgggagaag gatggcgagt tcagcgtgct gcagctggtg    660

ggcatgctgc ggggcatcgc agctggcatg aagtacctgg ccaacatgaa ctatgtgcac    720

cgtgacctgg ctgcccgcaa catcctctgtc aacagcaacc tggtctgcaa ggtgtctgac    780

tttggcctgt cccgcgtgct ggaggacgac ccgaggcca cctacaccac cagtggcggc    840

aagatcccca tccgctggac cgccccggag gccatttcct accggaagtt cacctctgcc    900
```

agcgacgtgt ggagctttgg cattgtcatg tgggaggtga tgacctatgg cgagcggccc 960

tactgggagt tgtccaacca cgaggtgatg aaagccatca atgatggctt ccggctcccc 1020

acacccatgg actgcccctc cgccatctac cagctcatga tgcagtgctg gcagcaggag 1080

cgtgcccgcc gccccaagtt cgctgacatc gtcagcatcc tggacaagct cattcgtgcc 1140

cctgactccc tcaagaccct ggctgacttt gacccccgcg tgtctatccg gctccccagc 1200

acgagcggct cggaggggct gcccttccgc acggtgtccg agtggctgga gtccatcaag 1260

atgcagcagt atacggagca cttcatggcg gccggctaca ctgccatcga gaaggtggtg 1320

cagatgacca acgacgacat caagaggatt ggggtgcggc tgcccggcca ccagaagcgc 1380

atcgcctaca gcctgctggg actcaaggac caggtgaaca ctgtggggat ccccatc 1437

<210> 36
<211> 479
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Predicted Protein Sequence

<400> 36

Met Lys Lys Ile Met Leu Val Phe Ile Thr Leu Ile Leu Val Ser Leu
1 5 10 15

Pro Ile Ala Gln Gln Thr Glu Ala Lys Asp Ala Ser Ala Phe Asn Lys
20 25 30

Glu Asn Ser Ile Ser Ser Met Ala Pro Pro Ala Ser Pro Pro Ala Ser
35 40 45

Pro Lys Thr Pro Ile Glu Lys Lys His Ala Asp Leu Glu His Arg Arg
50 55 60

Arg Lys Asn Gln Arg Ala Arg Gln Ser Pro Glu Asp Val Tyr Phe Ser
65 70 75 80

Lys Ser Glu Gln Leu Lys Pro Leu Lys Thr Tyr Val Asp Pro His Thr
85 90 95

Tyr Glu Asp Pro Asn Gln Ala Val Leu Lys Phe Thr Thr Glu Ile His
100 105 110

Pro Ser Cys Val Thr Arg Gln Lys Val Ile Gly Ala Gly Glu Phe Gly
115 120 125

```
Glu Val Tyr Lys Gly Met Leu Lys Thr Ser Ser Gly Lys Lys Glu Val
    130                 135                 140

Pro Val Ala Ile Lys Thr Leu Lys Ala Gly Tyr Thr Glu Lys Gln Arg
145                 150                 155                 160

Val Asp Phe Leu Gly Glu Ala Gly Ile Met Gly Gln Phe Ser His His
                165                 170                 175

Asn Ile Ile Arg Leu Glu Gly Val Ile Ser Lys Tyr Lys Pro Met Met
            180                 185                 190

Ile Ile Thr Glu Tyr Met Glu Asn Gly Ala Leu Asp Lys Phe Leu Arg
        195                 200                 205

Glu Lys Asp Gly Glu Phe Ser Val Leu Gln Leu Val Gly Met Leu Arg
    210                 215                 220

Gly Ile Ala Ala Gly Met Lys Tyr Leu Ala Asn Met Asn Tyr Val His
225                 230                 235                 240

Arg Asp Leu Ala Ala Arg Asn Ile Leu Val Asn Ser Asn Leu Val Cys
                245                 250                 255

Lys Val Ser Asp Phe Gly Leu Ser Arg Val Leu Glu Asp Asp Pro Glu
            260                 265                 270

Ala Thr Tyr Thr Thr Ser Gly Gly Lys Ile Pro Ile Arg Trp Thr Ala
        275                 280                 285

Pro Glu Ala Ile Ser Tyr Arg Lys Phe Thr Ser Ala Ser Asp Val Trp
    290                 295                 300

Ser Phe Gly Ile Val Met Trp Glu Val Met Thr Tyr Gly Glu Arg Pro
305                 310                 315                 320

Tyr Trp Glu Leu Ser Asn His Glu Val Met Lys Ala Ile Asn Asp Gly
                325                 330                 335

Phe Arg Leu Pro Thr Pro Met Asp Cys Pro Ser Ala Ile Tyr Gln Leu
            340                 345                 350

Met Met Gln Cys Trp Gln Gln Glu Arg Ala Arg Arg Pro Lys Phe Ala
        355                 360                 365

Asp Ile Val Ser Ile Leu Asp Lys Leu Ile Arg Ala Pro Asp Ser Leu
```

370 375 380

Lys Thr Leu Ala Asp Phe Asp Pro Arg Val Ser Ile Arg Leu Pro Ser
385 390 395 400

Thr Ser Gly Ser Glu Gly Val Pro Phe Arg Thr Val Ser Glu Trp Leu
405 410 415

Glu Ser Ile Lys Met Gln Gln Tyr Thr Glu His Phe Met Ala Ala Gly
420 425 430

Tyr Thr Ala Ile Glu Lys Val Val Gln Met Thr Asn Asp Asp Ile Lys
435 440 445

Arg Ile Gly Val Arg Leu Pro Gly His Gln Lys Arg Ile Ala Tyr Ser
450 455 460

Leu Leu Gly Leu Lys Asp Gln Val Asn Thr Val Gly Ile Pro Ile
465 470 475

<210> 37
<211> 1737
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Fusion protein sequence

<400> 37
ggtacctcct ttgattagta tattcctatc ttaaagttac ttttatgtgg aggcattaac 60

atttgttaat gacgtcaaaa ggatagcaag actagaataa agctataaag caagcatata 120

atattgcgtt tcatctttag aagcgaattt cgccaatatt ataattatca aaagagaggg 180

gtggcaaacg gtatttggca ttattaggtt aaaaaatgta gaaggagagt gaaacccatg 240

aaaaaaataa tgctagtttt tattacactt atattagtta gtctaccaat tgcgcaacaa 300

actgaagcaa aggatgcatc tgcattcaat aaagaaaatt caatttcatc catggcacca 360

ccagcatctc cgcctgcaag tcctaagacg ccaatcgaaa agaaacacgc ggatggatcc 420

gattataaag atgatgatga taaacacaga cgtagaaaaa atcaacgtgc tcgacaatcc 480

ccagaagatg tgtatttttc gaaaagtgaa caattaaaac cattaaaaac ttatgttgat 540

ccgcatacgt acgaagaccc aaatcaagca gtattaaaat ttacaacaga aatacaccca 600

agttgtgtta caagacaaaa agttattgga gcaggtgaat tcggagaggt atataaaggt 660

atgttaaaaa catcatcagg taaaaaagaa gttccggttg caattaaaac cttaaaggca 720

ggatatacag aaaaacagcg agttgatttt ttaggtgaag caggaattat gggtcaattt 780

agccatcata atattattcg tttggaagga gtaataagta aatataaacc aatgatgatt 840

attacagaat acatggaaaa cggtgcttta gataaatttt tacgtgaaaa ggatggtgaa 900

tttagtgttt tacaattggt tggtatgtta agaggaattg ctgcaggtat gaaatattta 960

gctaatatga attatgttca ccgtgatttg gcagcaagaa atatcctagt caattccaat 1020

ttagtatgta aagttagtga ttttggttta agcagagtat tagaagacga tccagaggca 1080

acctatacaa catcgggagg taaaattcct attcgttgga cagcaccaga agctatcagt 1140

taccgtaaat ttacaagtgc atcagacgtg tggagttttg ggattgtaat gtgggaagtt 1200

atgacatatg gagaaagacc atattgggaa ttaagtaatc atgaagttat gaaagcaatt 1260

aacgatggat ttagattacc aactccgatg gattgtccat ctgccattta tcaactaatg 1320

atgcaatgtt ggcaacaaga aagagcacga cgtccaaaat ttgcagatat tgttagtatt 1380

ttagacaaat taattcgtgc accagatagt ttaaaaactt tagcagactt tgatcctcgt 1440

gttagtattc gattaccaag tacgtcaggt tccgaaggag ttccatttcg cacagtctcc 1500

gaatggttgg aatcaattaa aatgcaacaa tacaccgaac actttatggc agcaggttac 1560

acagcaatcg aaaaagttgt tcaaatgaca aatgatgata ttaaacgtat tggagttaga 1620

ttaccaggcc accagaaacg tattgcatat tctttattag gtttaaaaga tcaagttaat 1680

accgtgggaa ttccaattga acaaaaatta atttccgaag aagacttata agagctc 1737

<210> 38
<211> 497
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Predicted fusion protein

<400> 38

Met Lys Lys Ile Met Leu Val Phe Ile Thr Leu Ile Leu Val Ser Leu
1 5 10 15

Pro Ile Ala Gln Gln Thr Glu Ala Lys Asp Ala Ser Ala Phe Asn Lys
20 25 30

Glu Asn Ser Ile Ser Ser Met Ala Pro Pro Ala Ser Pro Pro Ala Ser
35 40 45

Pro Lys Thr Pro Ile Glu Lys Lys His Ala Asp Gly Ser Asp Tyr Lys
50 55 60

Asp Asp Asp Asp Lys His Arg Arg Arg Lys Asn Gln Arg Ala Arg Gln

```
65                  70                  75                  80

Ser Pro Glu Asp Val Tyr Phe Ser Lys Ser Glu Gln Leu Lys Pro Leu
                85                  90                  95

Lys Thr Tyr Val Asp Pro His Thr Tyr Glu Asp Pro Asn Gln Ala Val
            100                 105                 110

Leu Lys Phe Thr Thr Glu Ile His Pro Ser Cys Val Thr Arg Gln Lys
        115                 120                 125

Val Ile Gly Ala Gly Glu Phe Gly Glu Val Tyr Lys Gly Met Leu Lys
    130                 135                 140

Thr Ser Ser Gly Lys Lys Glu Val Pro Val Ala Ile Lys Thr Leu Lys
145                 150                 155                 160

Ala Gly Tyr Thr Glu Lys Gln Arg Val Asp Phe Leu Gly Glu Ala Gly
                165                 170                 175

Ile Met Gly Gln Phe Ser His His Asn Ile Ile Arg Leu Glu Gly Val
            180                 185                 190

Ile Ser Lys Tyr Lys Pro Met Met Ile Ile Thr Glu Tyr Met Glu Asn
        195                 200                 205

Gly Ala Leu Asp Lys Phe Leu Arg Glu Lys Asp Gly Glu Phe Ser Val
    210                 215                 220

Leu Gln Leu Val Gly Met Leu Arg Gly Ile Ala Ala Gly Met Lys Tyr
225                 230                 235                 240

Leu Ala Asn Met Asn Tyr Val His Arg Asp Leu Ala Ala Arg Asn Ile
                245                 250                 255

Leu Val Asn Ser Asn Leu Val Cys Lys Val Ser Asp Phe Gly Leu Ser
            260                 265                 270

Arg Val Leu Glu Asp Asp Pro Glu Ala Thr Tyr Thr Thr Ser Gly Gly
        275                 280                 285

Lys Ile Pro Ile Arg Trp Thr Ala Pro Glu Ala Ile Ser Tyr Arg Lys
    290                 295                 300

Phe Thr Ser Ala Ser Asp Val Trp Ser Phe Gly Ile Val Met Trp Glu
305                 310                 315                 320
```

```
Val Met Thr Tyr Gly Glu Arg Pro Tyr Trp Glu Leu Ser Asn His Glu
                325                 330                 335

Val Met Lys Ala Ile Asn Asp Gly Phe Arg Leu Pro Thr Pro Met Asp
            340                 345                 350

Cys Pro Ser Ala Ile Tyr Gln Leu Met Met Gln Cys Trp Gln Gln Glu
        355                 360                 365

Arg Ala Arg Arg Pro Lys Phe Ala Asp Ile Val Ser Ile Leu Asp Lys
    370                 375                 380

Leu Ile Arg Ala Pro Asp Ser Leu Lys Thr Leu Ala Asp Phe Asp Pro
385                 390                 395                 400

Arg Val Ser Ile Arg Leu Pro Ser Thr Ser Gly Ser Glu Gly Val Pro
                405                 410                 415

Phe Arg Thr Val Ser Glu Trp Leu Glu Ser Ile Lys Met Gln Gln Tyr
            420                 425                 430

Thr Glu His Phe Met Ala Ala Gly Tyr Thr Ala Ile Glu Lys Val Val
        435                 440                 445

Gln Met Thr Asn Asp Asp Ile Lys Arg Ile Gly Val Arg Leu Pro Gly
    450                 455                 460

His Gln Lys Arg Ile Ala Tyr Ser Leu Leu Gly Leu Lys Asp Gln Val
465                 470                 475                 480

Asn Thr Val Gly Ile Pro Ile Glu Gln Lys Leu Ile Ser Glu Glu Asp
                485                 490                 495

Leu
```

<210> 39
<211> 1737
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Fusion protein construct

<400> 39
ggtacctcct ttgattagta tattcctatc ttaaagttac ttttatgtgg aggcattaac 60

atttgttaat gacgtcaaaa ggatagcaag actagaataa agctataaag caagcatata 120

atattgcgtt tcatctttag aagcgaattt cgccaatatt ataattatca aaagagaggg 180

gtggcaaacg gtatttggca ttattaggtt aaaaaatgta gaaggagagt gaaacccatg 240

aaaaaaatta tgttagtttt tattacatta attttagtta gtttaccaat tgcacaacaa 300

acagaagcaa aagatgcaag tgcatttaat aaagaaaata gtattagtag tatggcacca 360

ccagcaagtc caccagcaag tccaaaaaca ccaattgaaa aaaaacatgc agatggatcc 420

gattataaag acgatgatga taaacacaga cgtagaaaaa atcaacgtgc tcgacaatcc 480

ccagaagatg tgtatttttc gaaaagtgaa caattaaaac cattaaaaac ttatgttgat 540

ccgcatacgt acgaagaccc aaatcaagca gtattaaaat ttacaacaga aatacaccca 600

agttgtgtta caagacaaaa agttattgga gcaggtgaat tcggagaggt atataaaggt 660

atgttaaaaa catcatcagg taaaaaagaa gttccggttg caattaaaac cttaaaggca 720

ggatatacag aaaaacagcg agttgatttt ttaggtgaag caggaattat gggtcaattt 780

agccatcata atattattcg tttggaagga gtaataagta aatataaacc aatgatgatt 840

attacagaat acatggaaaa cggtgcttta gataaatttt tacgtgaaaa ggatggtgaa 900

tttagtgttt tacaattggt tggtatgtta agaggaattg ctgcaggtat gaaatattta 960

gctaatatga attatgttca ccgtgatttg gcagcaagaa atatcctagt caattccaat 1020

ttagtatgta aagttagtga ttttggttta agcagagtat tagaagacga tccagaggca 1080

acctatacaa catcgggagg taaaattcct attcgttgga cagcaccaga agctatcagt 1140

taccgtaaat ttacaagtgc atcagacgtg tggagttttg ggattgtaat gtgggaagtt 1200

atgacatatg gagaaagacc atattgggaa ttaagtaatc atgaagttat gaaagcaatt 1260

aacgatggat ttagattacc aactccgatg gattgtccat ctgccattta tcaactaatg 1320

atgcaatgtt ggcaacaaga aagagcacga cgtccaaaat ttgcagatat tgttagtatt 1380

ttagacaaat taattcgtgc accagatagt ttaaaaactt tagcagactt tgatcctcgt 1440

gttagtattc gattaccaag tacgtcaggt tccgaaggag ttccatttcg cacagtctcc 1500

gaatggttgg aatcaattaa aatgcaacaa tacaccgaac actttatggc agcaggttac 1560

acagcaatcg aaaaagttgt tcaaatgaca aatgatgata ttaaacgtat tggagttaga 1620

ttaccaggcc accagaaacg tattgcatat tctttattag gtttaaaaga tcaagttaat 1680

accgtgggaa ttccaattga acaaaaatta atttccgaag aagacttata agagctc 1737

<210> 40
<211> 497
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Predicted Fusion Protein

<400> 40

```
Met Lys Lys Ile Met Leu Val Phe Ile Thr Leu Ile Leu Val Ser Leu
1               5                  10                  15

Pro Ile Ala Gln Gln Thr Glu Ala Lys Asp Ala Ser Ala Phe Asn Lys
            20                  25                  30

Glu Asn Ser Ile Ser Ser Met Ala Pro Pro Ala Ser Pro Pro Ala Ser
        35                  40                  45

Pro Lys Thr Pro Ile Glu Lys Lys His Ala Asp Gly Ser Asp Tyr Lys
    50                  55                  60

Asp Asp Asp Asp Lys His Arg Arg Arg Lys Asn Gln Arg Ala Arg Gln
65                  70                  75                  80

Ser Pro Glu Asp Val Tyr Phe Ser Lys Ser Glu Gln Leu Lys Pro Leu
                85                  90                  95

Lys Thr Tyr Val Asp Pro His Thr Tyr Glu Asp Pro Asn Gln Ala Val
            100                 105                 110

Leu Lys Phe Thr Thr Glu Ile His Pro Ser Cys Val Thr Arg Gln Lys
        115                 120                 125

Val Ile Gly Ala Gly Glu Phe Gly Glu Val Tyr Lys Gly Met Leu Lys
    130                 135                 140

Thr Ser Ser Gly Lys Lys Glu Val Pro Val Ala Ile Lys Thr Leu Lys
145                 150                 155                 160

Ala Gly Tyr Thr Glu Lys Gln Arg Val Asp Phe Leu Gly Glu Ala Gly
                165                 170                 175

Ile Met Gly Gln Phe Ser His His Asn Ile Ile Arg Leu Glu Gly Val
            180                 185                 190

Ile Ser Lys Tyr Lys Pro Met Met Ile Ile Thr Glu Tyr Met Glu Asn
        195                 200                 205

Gly Ala Leu Asp Lys Phe Leu Arg Glu Lys Asp Gly Glu Phe Ser Val
    210                 215                 220
```

```
Leu Gln Leu Val Gly Met Leu Arg Gly Ile Ala Ala Gly Met Lys Tyr
225                 230                 235                 240

Leu Ala Asn Met Asn Tyr Val His Arg Asp Leu Ala Ala Arg Asn Ile
                245                 250                 255

Leu Val Asn Ser Asn Leu Val Cys Lys Val Ser Asp Phe Gly Leu Ser
            260                 265                 270

Arg Val Leu Glu Asp Asp Pro Glu Ala Thr Tyr Thr Thr Ser Gly Gly
        275                 280                 285

Lys Ile Pro Ile Arg Trp Thr Ala Pro Glu Ala Ile Ser Tyr Arg Lys
    290                 295                 300

Phe Thr Ser Ala Ser Asp Val Trp Ser Phe Gly Ile Val Met Trp Glu
305                 310                 315                 320

Val Met Thr Tyr Gly Glu Arg Pro Tyr Trp Glu Leu Ser Asn His Glu
                325                 330                 335

Val Met Lys Ala Ile Asn Asp Gly Phe Arg Leu Pro Thr Pro Met Asp
            340                 345                 350

Cys Pro Ser Ala Ile Tyr Gln Leu Met Met Gln Cys Trp Gln Gln Glu
        355                 360                 365

Arg Ala Arg Arg Pro Lys Phe Ala Asp Ile Val Ser Ile Leu Asp Lys
    370                 375                 380

Leu Ile Arg Ala Pro Asp Ser Leu Lys Thr Leu Ala Asp Phe Asp Pro
385                 390                 395                 400

Arg Val Ser Ile Arg Leu Pro Ser Thr Ser Gly Ser Glu Gly Val Pro
                405                 410                 415

Phe Arg Thr Val Ser Glu Trp Leu Glu Ser Ile Lys Met Gln Gln Tyr
            420                 425                 430

Thr Glu His Phe Met Ala Ala Gly Tyr Thr Ala Ile Glu Lys Val Val
        435                 440                 445

Gln Met Thr Asn Asp Asp Ile Lys Arg Ile Gly Val Arg Leu Pro Gly
    450                 455                 460
```

His Gln Lys Arg Ile Ala Tyr Ser Leu Leu Gly Leu Lys Asp Gln Val
465 470 475 480

Asn Thr Val Gly Ile Pro Ile Glu Gln Lys Leu Ile Ser Glu Glu Asp
485 490 495

Leu

<210> 41
<211> 1716
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Fusion protein construct

<400> 41
ggtacctcct ttgattagta tattcctatc ttaaagttac ttttatgtgg aggcattaac 60

atttgttaat gacgtcaaaa ggatagcaag actagaataa agctataaag caagcatata 120

atattgcgtt tcatctttag aagcgaattt cgccaatatt ataattatca aaagagaggg 180

gtggcaaacg gtatttggca ttattaggtt aaaaaatgta gaaggagagt gaaacccatg 240

gcatacgaca gtcgttttga tgaatgggta cagaaactga aagaggaaag ctttcaaaac 300

aatacgtttg accgccgcaa atttattcaa ggagcgggga agattgcagg actttctctt 360

ggattaacga ttgcccagtc ggttggggcc tttggatccg attataaaga tgatgatgat 420

aaacacagac gtagaaaaaa tcaacgtgct cgacaatccc cagaagatgt gtatttttcg 480

aaaagtgaac aattaaaacc attaaaaact tatgttgatc cgcatacgta cgaagaccca 540

aatcaagcag tattaaaatt tacaacagaa atacacccaa gttgtgttac aagacaaaaa 600

gttattggag caggtgaatt cggagaggta tataaaggta tgttaaaaac atcatcaggt 660

aaaaaagaag ttccggttgc aattaaaacc ttaaggcag gatatacaga aaaacagcga 720

gttgattttt taggtgaagc aggaattatg ggtcaattta gccatcataa tattattcgt 780

ttggaaggag taataagtaa atataaacca atgatgatta ttacagaata catggaaaac 840

ggtgctttag ataaattttt acgtgaaaag gatggtgaat ttagtgtttt acaattggtt 900

ggtatgttaa gaggaattgc tgcaggtatg aaatatttag ctaatatgaa ttatgttcac 960

cgtgatttgg cagcaagaaa tatcctagtc aattccaatt tagtatgtaa agttagtgat 1020

tttggtttaa gcagagtatt agaagacgat ccagaggcaa cctatacaac atcgggaggt 1080

aaaattccta ttcgttggac agcaccagaa gctatcagtt accgtaaatt tacaagtgca 1140

tcagacgtgt ggagttttgg gattgtaatg tgggaagtta tgacatatgg agaaagacca 1200

```
tattgggaat taagtaatca tgaagttatg aaagcaatta acgatggatt tagattacca   1260

actccgatgg attgtccatc tgccatttat caactaatga tgcaatgttg gcaacaagaa   1320

agagcacgac gtccaaaatt tgcagatatt gttagtattt tagacaaatt aattcgtgca   1380

ccagatagtt taaaaacttt agcagacttt gatcctcgtg ttagtattcg attaccaagt   1440

acgtcaggtt ccgaaggagt tccatttcgc acagtctccg aatggttgga atcaattaaa   1500

atgcaacaat acaccgaaca ctttatggca gcaggttaca cagcaatcga aaaagttgtt   1560

caaatgacaa atgatgatat taaacgtatt ggagttagat taccaggcca ccagaaacgt   1620

attgcatatt ctttattagg tttaaaagat caagttaata ccgtgggaat tccaattgaa   1680

caaaaattaa tttccgaaga agacttataa gagctc                             1716
```

<210> 42
<211> 490
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Predicted fusion protein

<400> 42

```
Met Ala Tyr Asp Ser Arg Phe Asp Glu Trp Val Gln Lys Leu Lys Glu
1               5                  10                  15

Glu Ser Phe Gln Asn Asn Thr Phe Asp Arg Arg Lys Phe Ile Gln Gly
            20                  25                  30

Ala Gly Lys Ile Ala Gly Leu Ser Leu Gly Leu Thr Ile Ala Gln Ser
        35                  40                  45

Val Gly Ala Phe Gly Ser Asp Tyr Lys Asp Asp Asp Asp Lys His Arg
    50                  55                  60

Arg Arg Lys Asn Gln Arg Ala Arg Gln Ser Pro Glu Asp Val Tyr Phe
65                  70                  75                  80

Ser Lys Ser Glu Gln Leu Lys Pro Leu Lys Thr Tyr Val Asp Pro His
                85                  90                  95

Thr Tyr Glu Asp Pro Asn Gln Ala Val Leu Lys Phe Thr Thr Glu Ile
            100                 105                 110

His Pro Ser Cys Val Thr Arg Gln Lys Val Ile Gly Ala Gly Glu Phe
        115                 120                 125
```

```
Gly Glu Val Tyr Lys Gly Met Leu Lys Thr Ser Ser Gly Lys Lys Glu
    130                 135                 140

Val Pro Val Ala Ile Lys Thr Leu Lys Ala Gly Tyr Thr Glu Lys Gln
145                 150                 155                 160

Arg Val Asp Phe Leu Gly Glu Ala Gly Ile Met Gly Gln Phe Ser His
                165                 170                 175

His Asn Ile Ile Arg Leu Glu Gly Val Ile Ser Lys Tyr Lys Pro Met
            180                 185                 190

Met Ile Ile Thr Glu Tyr Met Glu Asn Gly Ala Leu Asp Lys Phe Leu
        195                 200                 205

Arg Glu Lys Asp Gly Glu Phe Ser Val Leu Gln Leu Val Gly Met Leu
    210                 215                 220

Arg Gly Ile Ala Ala Gly Met Lys Tyr Leu Ala Asn Met Asn Tyr Val
225                 230                 235                 240

His Arg Asp Leu Ala Ala Arg Asn Ile Leu Val Asn Ser Asn Leu Val
                245                 250                 255

Cys Lys Val Ser Asp Phe Gly Leu Ser Arg Val Leu Glu Asp Asp Pro
            260                 265                 270

Glu Ala Thr Tyr Thr Thr Ser Gly Gly Lys Ile Pro Ile Arg Trp Thr
        275                 280                 285

Ala Pro Glu Ala Ile Ser Tyr Arg Lys Phe Thr Ser Ala Ser Asp Val
    290                 295                 300

Trp Ser Phe Gly Ile Val Met Trp Glu Val Met Thr Tyr Gly Glu Arg
305                 310                 315                 320

Pro Tyr Trp Glu Leu Ser Asn His Glu Val Met Lys Ala Ile Asn Asp
                325                 330                 335

Gly Phe Arg Leu Pro Thr Pro Met Asp Cys Pro Ser Ala Ile Tyr Gln
            340                 345                 350

Leu Met Met Gln Cys Trp Gln Gln Glu Arg Ala Arg Arg Pro Lys Phe
        355                 360                 365

Ala Asp Ile Val Ser Ile Leu Asp Lys Leu Ile Arg Ala Pro Asp Ser
```

```
    370                 375                 380

Leu Lys Thr Leu Ala Asp Phe Asp Pro Arg Val Ser Ile Arg Leu Pro
385                 390                 395                 400

Ser Thr Ser Gly Ser Glu Gly Val Pro Phe Arg Thr Val Ser Glu Trp
                405                 410                 415

Leu Glu Ser Ile Lys Met Gln Gln Tyr Thr Glu His Phe Met Ala Ala
            420                 425                 430

Gly Tyr Thr Ala Ile Glu Lys Val Val Gln Met Thr Asn Asp Asp Ile
        435                 440                 445

Lys Arg Ile Gly Val Arg Leu Pro Gly His Gln Lys Arg Ile Ala Tyr
    450                 455                 460

Ser Leu Leu Gly Leu Lys Asp Gln Val Asn Thr Val Gly Ile Pro Ile
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Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu
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<223> Description of Artificial Sequence: Fusion Protein Construct

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<213> Listeria monocytogenes

<400> 44

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Pro Ile Ala Gln Gln Thr Glu Ala Lys Asp
20 25

<210> 45
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<212> PRT
<213> Listeria monocytogenes

<400> 45

Met Thr Asp Lys Lys Ser Glu Asn Gln Thr Glu Lys Thr Glu Thr Lys
1 5 10 15

Glu Asn Lys Gly Met Thr Arg Arg Glu Met Leu Lys Leu Ser Ala Val
20 25 30

Ala Gly Thr Gly Ile Ala Val Gly Ala Thr Gly Leu Gly Thr Ile Leu
35 40 45

Asn Val Val Asp Gln Val Asp Lys Ala Leu Thr
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<210> 46
<211> 53
<212> PRT
<213> Bacillus subtillus

<400> 46

Met Ala Tyr Asp Ser Arg Phe Asp Glu Trp Val Gln Lys Leu Lys Glu
1 5 10 15

Glu Ser Phe Gln Asn Asn Thr Phe Asp Arg Arg Lys Phe Ile Gln Gly
20 25 30

Ala Gly Lys Ile Ala Gly Leu Ser Leu Gly Leu Thr Ile Ala Gln Ser
35 40 45

Val Gly Ala Phe Gly

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<223> Description of Artificial Sequence: Primer

<400> 48
acataatcag tccaaagtag atgc 24

<210> 49
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<400> 49
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<210> 50
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<212> DNA
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<223> Description of Artificial Sequence: Primer

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<210> 51
<211> 24
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<400> 51
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<210> 52
<211> 8
<212> PRT
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<210> 53
<211> 30
<212> DNA
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<400> 53
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<210> 54
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<400> 54

Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu
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<210> 55
<211> 9
<212> PRT
<213> Mus sp.

<400> 55

Ser Pro Ser Tyr Val Tyr His Gln Phe
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<210> 56
<211> 9
<212> PRT
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<223> Description of Artificial Sequence: Epitope

<400> 56

Ser Pro Ser Tyr Ala Tyr His Gln Phe
1 5

<210> 57
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<212> DNA
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<220>
<223> Description of Artificial Sequence: Primer

<400> 57
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caatggatcc ctcgagatca taatttactt catccc 36

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<210> 61
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<220>
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<400> 61
cgattcccct agttatgttt accaccaatt tgctgca 37

<210> 62
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gcaaattggt ggtaaacata actaggggaa t 31

<210> 63
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<210> 64
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<220>
<223> Description of Artificial Sequence: Primer

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<210> 66
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<212> DNA
<213> Listeria monocytogenes

<400> 67
aaggagagtg aaacccatg 19

<210> 68
<211> 240
<212> DNA
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<400> 68
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<210> 69
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atattgcgtt tcatctttag aagcgaattt cgccaatatt ataattatca aaagagaggg 180

gtggcaaacg gtatttggca ttattaggtt aaaaaatgta gaaggagagt gaaacccatg 240

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<211> 5
<212> PRT
<213> Listeria monocytogenes

<400> 70

Thr Glu Ala Lys Asp
1 5

<210> 71

<211> 5
<212> PRT
<213> Listeria monocytogenes

<400> 71

Asp Lys Ala Leu Thr
1 5

<210> 72
<211> 5
<212> PRT
<213> Bacillus subtillus

<400> 72

Val Gly Ala Phe Gly
1 5

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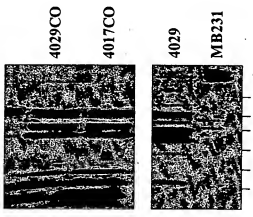


Expected mass of EphA2 proteins

Full-length: 114 kDa

EphA2 CO: 52 kDa

EphA2 EX2: 62 kDa

Ab: Rabbit anti-EphA2 (carboxy terminus-specific) polyclonal antibody

Figure 1

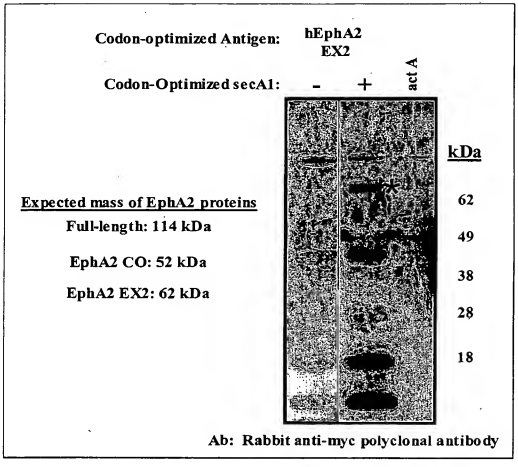

Codon-optimized Antigen:
hEphA2 EX2
Codon-Optimized secA1:
-
+
act A
kDa
62
49
38
28
18
Expected mass of EphA2 proteins
Full-length: 114 kDa
EphA2 CO: 52 kDa
EphA2 EX2: 62 kDa
Ab: Rabbit anti-myc polyclonal antibody

Figure 2

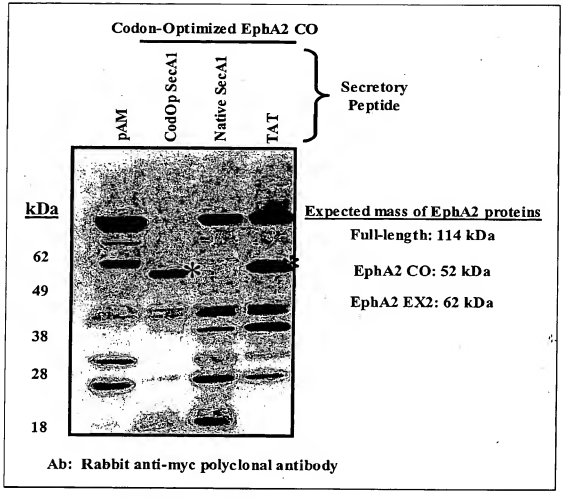
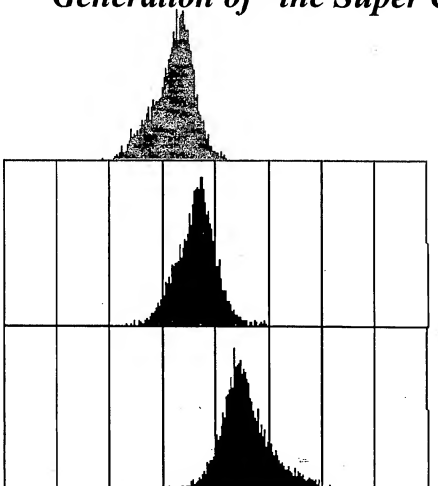

Codon-Optimized EphA2 CO
pAM
CodOp SecA1
Native SecA1
TAT
Secretory Peptide
kDa
62
49
38
28
18
Expected mass of EphA2 proteins
Full-length: 114 kDa
EphA2 CO: 52 kDa
EphA2 EX2: 62 kDa
Ab: Rabbit anti-myc polyclonal antibody

Figure 3

Figure 4

Human EphA2 Expression in CT26 Subclones

Generation of "the Super Clone"



A8-B5 (mean = 45.0)
Average

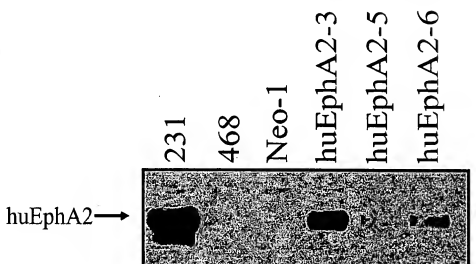
B7-D10 (mean = 73.18)
Medium

B7-E6 (mean = 233.0)
Super Clone!

• Subclones of transfected clones obtained by FACS sorting into 96 well plates

Figure 5

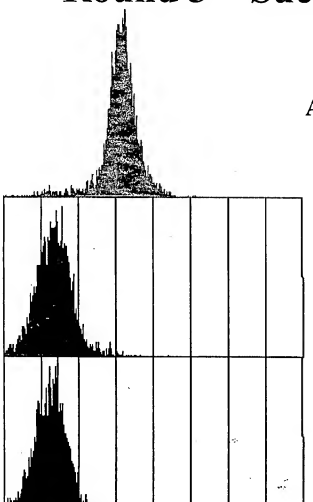
Human EphA2 Protein Expression in CT26 Murine Colon Carcinoma Cells Following FACS Sorting



•pooled populations of transfected cells sorted by FACS

Figure 6

Human EphA2 Protein Expression in B16F10 Cells Round 3 – Success!




pcDNA4
MB231
pcDNA4-huEphA2
kDa
188
62
Expected mass of EphA2 proteins
Full-length: 114 kDa
EphA2 CO: 52 kDa
EphA2 EX2: 62 kDa
Ab: Rabbit anti-EphA2 (carboxy terminus-specific) polyclonal antibody

Figure 7

Figure 8A

Effect of *L. monocytogenes* Expressing ECD of huEphA2 on CT26 Tumor Growth

Therapeutic Study

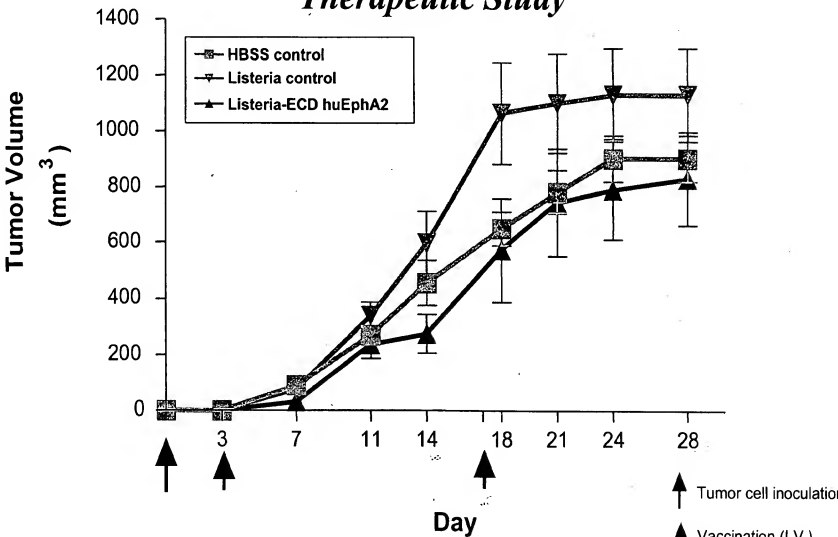


Figure 8B

Effect of *L. monocytogenes* Expressing ECD of huEphA2 on CT26 Tumor Growth

Therapeutic Study

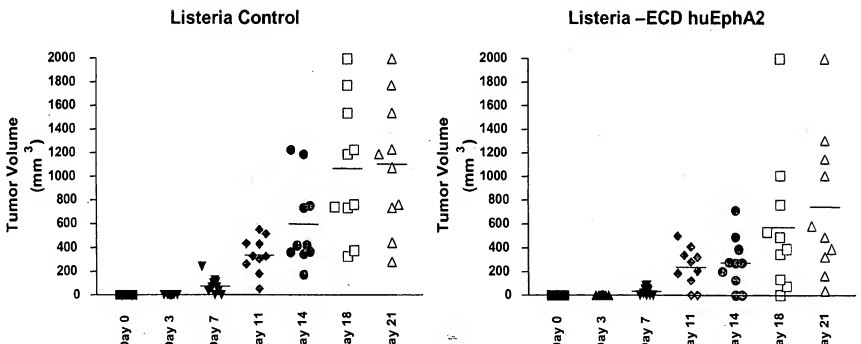


Figure 8C

Animal Survival in CT26 Experimental Lung Metastases Model

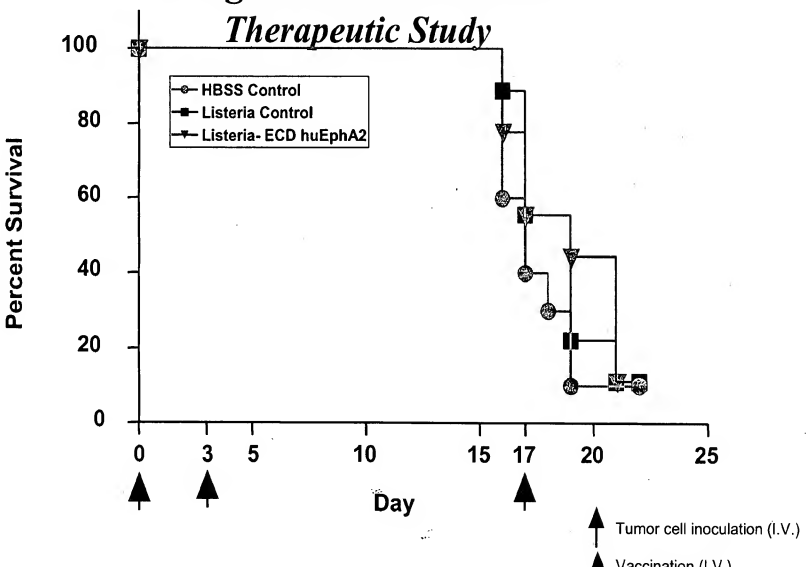


Figure 9A

Effect of *L. monocytogenes* Expressing ECD of huEphA2 on CT26 huEphA2 Tumor Growth

Prevention Study

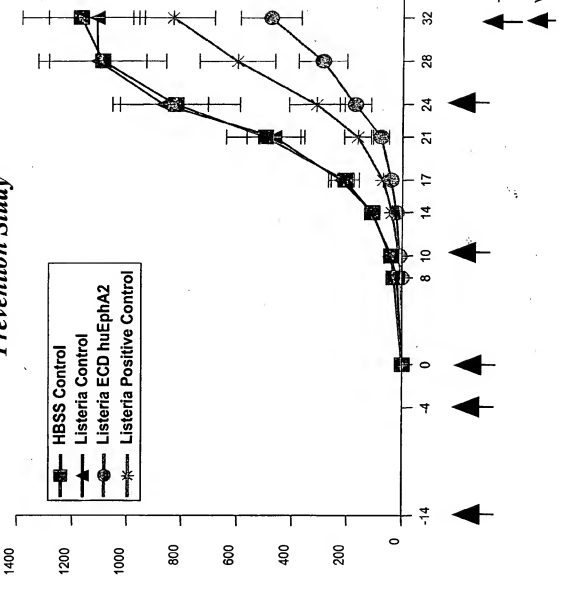


Figure 9B

Effect of *L. monocytogenes* Expressing ECD of huEphA2 on CT26 huEphA2 Tumor Growth
Prevention Study

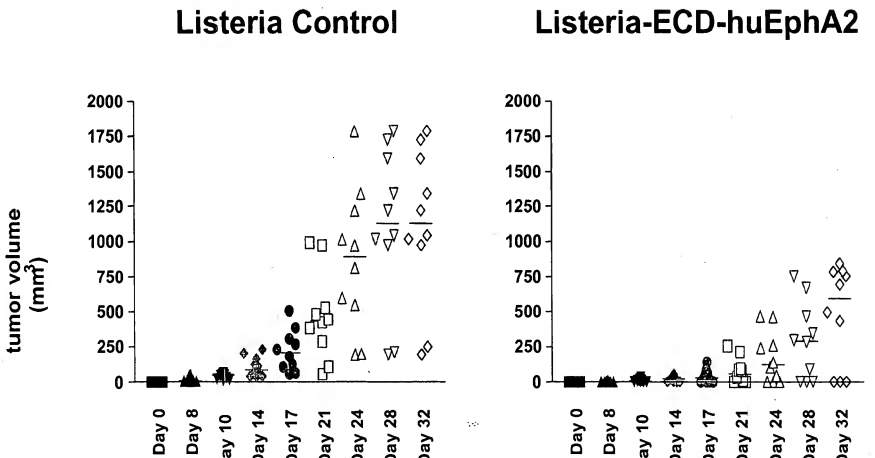


Figure 9C

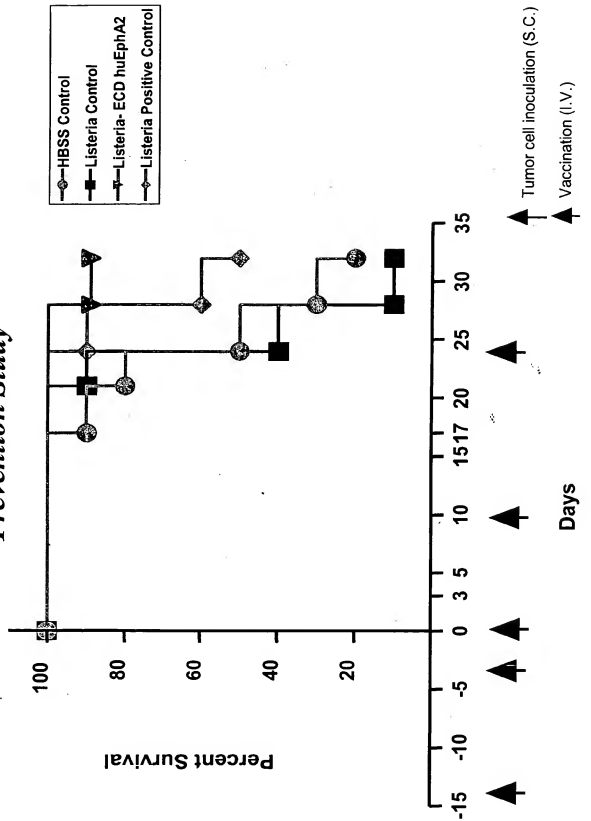
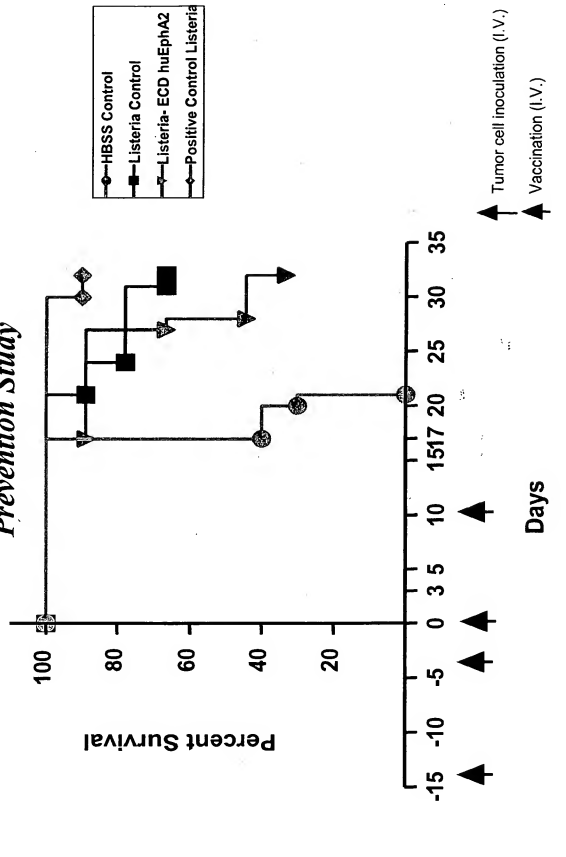

Animal Survival in CT26 huEphA2 S.C. Model
Prevention Study
Percent Survival
100
80
60
40
20
-15
-10
-5
0
3
5
10
15
17
20
25
30
35
Days
HBSS Control
Listeria Control
Listeria- ECD huEphA2
Listeria Positive Control
Tumor cell inoculation (S.C.)
Vaccination (I.V.)

Figure 9D


Animal Survival in CT26 huEphA2
Lung Metastases Model
Prevention Study
Percent Survival
100
80
60
40
20
-15 -10 -5 0 3 5 10 15 17 20 25 30 35
Days
HBSS Control
Listeria Control
Listeria- ECD huEphA2
Positive Control Listeria
Tumor cell inoculation (I.V.)
Vaccination (I.V.)

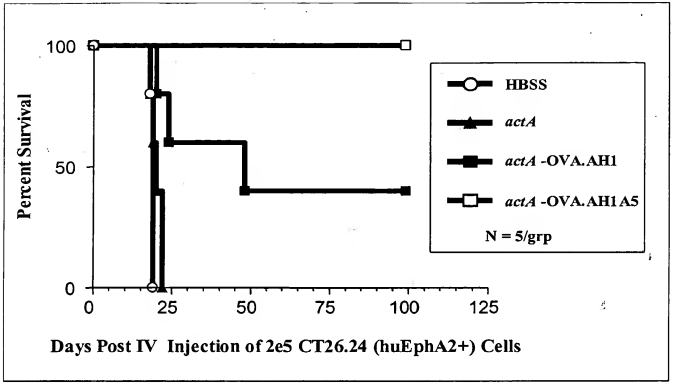

100
50
0
Percent Survival
0
25
50
75
100
125
Days Post IV Injection of 2e5 CT26.24 (huEphA2+) Cells
HBSS
actA
actA -OVA.AH1
actA -OVA.AH1A5
N = 5/grp

Figure 10

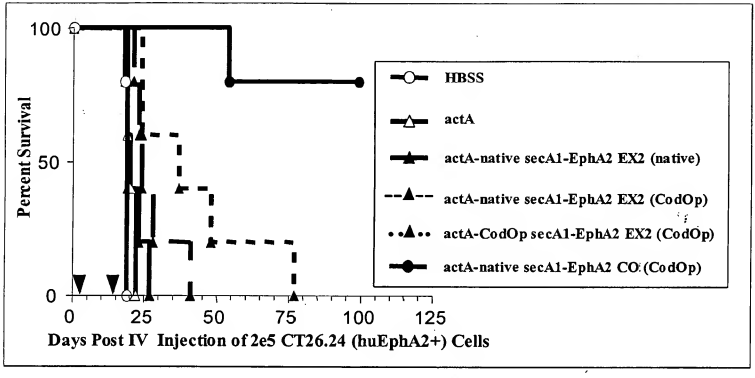

100
50
0
Percent Survival
0
25
50
75
100
125
Days Post IV Injection of 2e5 CT26.24 (huEphA2+) Cells
HBSS
actA
actA-native secA1-EphA2 EX2 (native)
actA-native secA1-EphA2 EX2 (CodOp)
actA-CodOp secA1-EphA2 EX2 (CodOp)
actA-native secA1-EphA2 CO (CodOp)

Figure 11

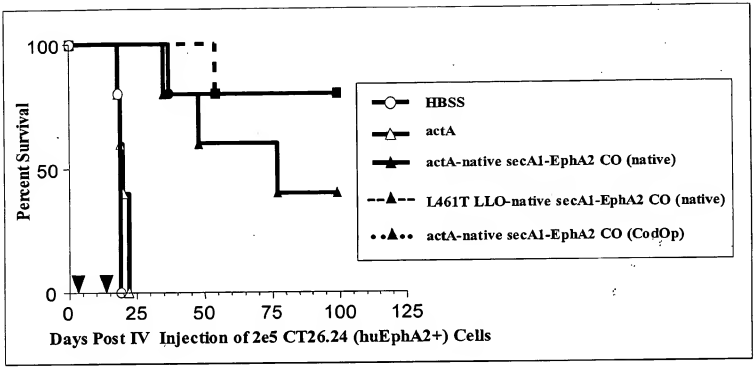

100
50
0
Percent Survival
0
25
50
75
100
125
Days Post IV Injection of 2e5 CT26.24 (huEphA2+) Cells
HBSS
actA
actA-native secA1-EphA2 CO (native)
L461T LLO-native secA1-EphA2 CO (native)
actA-native secA1-EphA2 CO (CodOp)

Figure 12

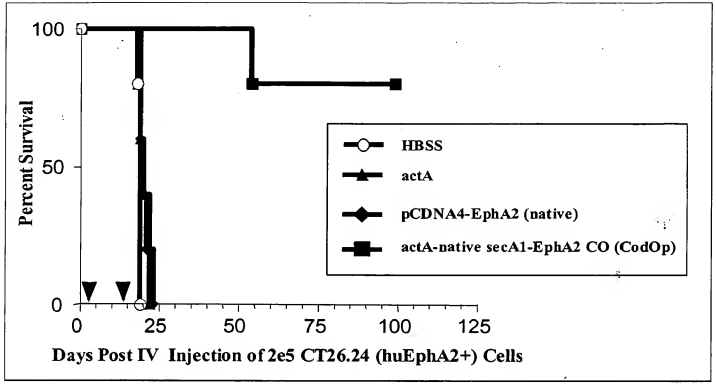

100
50
0
Percent Survival
0
25
50
75
100
125
Days Post IV Injection of 2e5 CT26.24 (huEphA2+) Cells
HBSS
actA
pCDNA4-EphA2 (native)
actA-native secA1-EphA2 CO (CodOp)

Figure 13

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